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TITLE OF THESIS..CEREBRAL CIRCULATORY RESPONSES TO SUBARACHNOID HEMORRHAGE IN THE RHESUS MONKEY

UNIVERSITY......ALBERTA

DEGREE FOR WHICH THESIS WAS PRESENTED......Ph.D

YEAR THIS DEGREE GRANTED......1976

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# CEREBRAL CIRCULATORY RESPONSES TO SUBARACHNOID HEMORRHAGE IN THE RHESUS MONKEY

by

KENNETH CAMERON PETRUK ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY
IN
EXPERIMENTAL SURGERY

DEPARTMENT: SURGERY

EDMONTON, ALBERTA

FALL, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled CEREBRAL CIRCULATORY RESPONSES TO SUBARACHNOID HEMORRHAGE IN THE RHESUS MONKEY submitted by KENNETH CAMERON PETRUK in partial fulfilment of the requirements for the degree of Doctor of Philosophy in EXPERIMENTAL SURGERY ( NEUROSURGERY)

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# ABSTRACT

The effect of induced subarachnoid hemorrhage (SAH) on cerebral blood flow (CBF) and cerebral arterial reactivity was investigated in lightly anesthetized rhesus monkeys. Cerebral blood flow (partial hemispheric and regional) was measured by the intra-arterial radioisotope tissue clearance method of Ingvar and Lassen. Concurrent serial angiographical studies were performed during a 3-hour period after induction of SAH. Other physiological parameters monitored throughout the experimental period included blood pressure, heart rate, EKG, arterial blood gases and pH, EEG and intracranial pressure. The changes observed were subsequently correlated with the clinical (neurological) state of the animals upon reversal of anesthesia.

Subarachnoid hemorrhage caused a significant reduction in the cerebral blood flow in 75-80 percent of animals. The reduction in CBF occurred immediately after the onset of SAH and remained low for the duration of the experimental period. Generalized angiographical vasospasm was present in 100 percent of animals subjected to SAH. The degree of vasospasm present in animals exhibiting reduced cerebral perfusion was not significantly different from animals not exhibiting decrease in CBF.

Cerebral insult (SAH) and traumatic spasm of the internal carotid artery (TSICA) was associated with a decreased CBF response to induced hypocapnia and hypercapnia. Severe hypercapnia ($PaCO_2$ values of 60 mm Hg to 65 mm Hg) caused marked increased in cerebral perfusion even though vasospasm was not alleviated.

These investigations support the hypothesis that cerebral

blood flow studies adequately reflect the status of the cerebral circulation and neurological function after subarachnoid hemorrhage.

Furthermore, it is concluded that angiographical vasospasm of the large capacitance vessels after subarachnoid hemorrhage does not necessarily indicate impairment of cerebral perfusion and function.

# ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. B.K.A. Weir who suggested the topic for this investigation, constantly guided its course, provided valuable advice and participated actively in its execution.

I am gratefully indebted to Dr. T.R. Overton for providing use of the excellent facilities and instrumentation of the Biomedical Engineering laboratory and for his valuable suggestions, recommendations, instruction and counselling in the field of medical physics.

The writer would also like to take this opportunity to thank the following:

Mr. Michael Marriott, B.Sc., (Eng.) for his generous contributions in computer analysis and programming.

Dr. M. Grace and his assistants for their assistance in statistical analysis.

Mrs. Joan Harvey for her willing and untiring technical assistance during the experimental procedures.

Dr. D. Menon for his assistance with the mathematical theories of cerebral blood flow calculation.

Dr. J.R. MacIntyre for providing the anesthetic equipment and for his assistance in the field of animal anesthesiology.

Dr. J.D.R. Miller for the use of the radiologic equipment and his assistance in x-ray interpretation.

Mr. L. Friedenberg for his technical instruction and assistance.

Dr. D. Secord and his staff for the care and assistance in handling the animals.

Mrs. Elaine Finley for typing this thesis and several publication manuscripts.

The University of Alberta photographic department for preparing prints for this thesis.

The financial assistance provided by the Medical Research Council of Canada Dr. B. K. A. Weir - (55 - 42191 MRC 4054) and Dr. T. R. Overton - (55 - 42137 MRC MA3352) is very gratefully acknowledged.

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# CHAPTER ONE

## INTRODUCTION

The pathophysiologic mechanisms involved in the production of decreased cerebral perfusion and function following subarachnoid hemorrhage (SAH) in man remain to be fully elucidated. Although cerebral arterial spasm has been implicated as the principal cause of morbidity and mortality after aneurysmal rupture, knowledge regarding the mechanism of initiation, propagation and prolongation of pathological vasoconstriction is incomplete. While marked vasospasm may appear to diminish cerebral blood flow (CBF), recent clinical and experimental studies have failed to show a direct correlation between vasospasm, cerebral ischemia and development of neurological sequelae.

Interest continues in developing improved models, comparable to the clinical phenomenon, for studying cerebral arterial spasm. Utilizing improved angiographical techniques, pathological vasoconstriction has been induced by avulsion or puncture of a major cerebral artery and by injection of the whole autogeneous blood into subarachnoid spaces at the base of the brain. However, it seems doubtful that the radiological picture alone can serve as an adequate prognostic indicator regarding cerebral perfusion and neurological function when vasospasm exists.

The initial study presented here (study A) was designed to investigate the acute effect of induced subarachnoid hemorrhage on cerebral blood flow determined by the $^{133}$Xenon ($^{133}$Xe) intra-arterial technique in the rhesus monkey. Changes in cerebral blood flow after induced SAH were correlated with the neurological condition of the animals immediately following each experiment. In addition, the effect of subarachnoid acidic saline injection on cerebral perfusion was studied in several animals. Cardiovascular, electroencephalographic and intracranial pressure changes in response to subarachnoid hemorrhage and subarachnoid saline injection were simultaneously monitored.

The second study (study B) was undertaken to determine whether reduced cerebral perfusion observed after induced subarachnoid hemorrhage was primarily due to development of cerebral arterial spasm. This study concurrently investigated changes in angiographical arterial caliber and regional cerebral blood flow and the changes observed were subsequently compared to the clinical and neurological state of the animals following termination of each experiment. In addition, the effect of induced subdural hemorrhage on regional cerebral blood flow, angiographical caliber of cerebral arteries and neurological state of the animals was investigated.

The final study (study C) was designed to concurrently investigate regional cerebral perfusion (rCBF) responses and intradural vessel reactivity (angiography) to graded carbon dioxide tension changes in control monkeys and monkeys subjected to induced subarachnoid hemorrhage and traumatic internal carotid artery spasm. The observed changes were statistically analyzed and conclusions were formulated.

# CHAPTER TWO

## HISTORICAL REVIEW

### I. PHYSIOLOGY OF CEREBRAL CIRCULATION

As in all perfused organs, blood flow through the brain tissue is directly proportional to the driving perfusion pressure and inversely proportional to the cerebrovascular resistance, i.e.,

$$\text{Flow} = \frac{\text{Cerebral Perfusion Pressure}}{\text{Cerebrovascular Resistance}}$$

#### A. The Cerebral Perfusion Pressure

The pressure of the blood perfusing the brain is the difference between the mean arterial blood pressure (MABP) minus the mean intracranial pressure (ICP) and the intracranial venous pressure (ICVP):

$$\text{CPP} = \text{MABP} - (\text{MICP} + \text{MICVP})$$

As the cerebral venous pressure is comparatively low (approximately 5 mm Hg), it can be assumed to contribute little to the regulation of cerebral blood flow under normal circumstances. Moyer, et al. (1) studied the effect of increased cerebral venous pressure to 18 mm Hg in normal subjects and found no alteration in cerebral perfusion. Cerebral venous pressure, however, becomes an important factor in maintaining adequate cerebral perfusion during positive gravitational stress. Henry, et al. (2) demonstrated that at 4-5 G, cerebral arterial pressure dropped to only a few millimeters mercury but cerebral perfusion was maintained at a constant level because cerebral venous pressure fell to nearly minus 60 mm Hg.

Recent studies (3,4,5) have emphasized the effect of increased intracranial pressure (ICP) on cerebral blood flow (CBF) through alteration in cerebral perfusion pressure. Cerebral perfusion pressure was calculated as the difference between the mean arterial blood pressure and the mean intracranial pressure. Zwetnow (4) and Haggendal (5) have shown that CBF in the dog remained constant over a wide range of levels of increased ICP (0-100 mmHg). Cerebral blood flow did not decrease until the cerebral perfusion pressure fell below 40 mm Hg. This findings was confirmed by Johnson (6) in baboons and by Miller (7,8) in dogs.

Under normal physiological conditions, the major factor determining cerebral perfusion pressure is the cerebral arterial pressure. Cerebral blood flow studies in man have shown a remarkably constant flow over a wide range of perfusion pressures. Experimental and clinical studies (9,10,11,12,13,14,15,16) have demonstrated that CBF is kept constant over a mean arterial pressure range varying from 50-80 mm Hg and 225 mm Hg or more. Not until the mean arterial pressure falls below the range 50 mm Hg - 80 mm Hg does CBF decline. These studies suggested the presence of an autoregulation of cerebrovascular tone and thus cerebral blood flow in response to changes in cerebral perfusion pressure.

Autoregulation represents one of the fundamental mechanisms which controls cerebral blood flow and is defined as the inherent property of the brain to maintain a constant blood flow despite changes in cerebral perfusion pressure. This phenomenon was first described by Fog (9) and Forbes (13) and has been recently confirmed by inert tracer CBF studies.

The mechanism of autoregulation has not been established and three theories regarding its pathogenesis have been proposed.

(a) Myogenic Theory:

The studies of Bayliss (17) and Folkow (18,19) in the peripheral vascular system led to the consideration of a myogenic mechanism in the cerebral circulation. According to this hypothesis the resistance vessels are considered to have a high degree of resting vasomotor tone due mainly to smooth muscle contraction in the walls. An increase in intravascular pressure stimulates a further increase in tone by first stretching the muscle causing a reactive shortening of radial fibers and a reduction of vascular radius. A decrease in intraluminal pressure has the opposite effect causing vasodilation. The effective pressure stimulus is not the intraluminal pressure per se but is the difference between intravascular and extravascular pressure, i.e., the transmural pressure. This theory assumes that neurogenic and metabolic factors play little or no part in the maintenance of autoregulation.

(b) Metabolic Theory:

The metabolic theory postulates that the basal tone of the cerebral vessels is secondarily influenced by the action of biochemical vasoactive substances (cerebral metabolites, carbon dioxide and oxygen). Meyer, _et_ _al_. (20) observed that reduction in blood pressure and blood flow resulted in decreased tissue $PO_2$ and increased tissue $PCO_2$ which in turn led to a decrease in cerebrovascular resistance and an increase in blood flow. These events were primarily due to the sensitivity of vascular smooth muscle cells to changes in $PCO_2$ and pH. Gotoh, _et_ _al_. (21) demonstrated that the change in tissue $PCO_2$ exerted its effect on vascular smooth muscle by reducing the pH within smooth muscle cells.

### (c) Neurogenic Theory:

Evidence concerning the importance of neurogenic influences on autoregulatory response has been rapidly accumulating (22,23,24, 25,26). Pathways of the autonomic nervous system from brain stem centers have been established and rich autonomic innervation of the cerebral vessels has been confirmed (27,28,29,30,31). While neurogenic factors have been previously assumed to have an insignificant influence on the mechanism of autoregulation (32,33), recent evidence has demonstrated that neurogenic factors participate in the control of autoregulation (24,25,26). Meyer, et al. (34) have demonstrated impairment of autoregulation with preserved chemical vasomotor control of CBF in patients with brain stem lesions and these authors hypthesized that a neurogenic mechanism via the autonomic nervous system was responsible for the control of cerebral autoregulation.

## B. Cerebrovascular Resistance

Resistance is directly proportional to the length of the vessel (which is relatively fixed) and inversely proportional to the fourth power of the radius (35). Thus changes in diameter or tone of the vessel plays a major role in altering cerebrovascular resistance (CVR). The factors influencing cerebrovascular tone are classified as either chemical (metabolic) or neurogenic.

### (a) Metabolic Control of Cerebral Blood Flow

Studies in animals and man during seizures, sensory stimulation or arousal demonstrated an increase in cerebral metabolism causing a secondary or indirect increase in cerebral blood flow ( 36,37,38). It became apparent that local increases in brain tissue $PCO_2$ caused by increased metabolism were responsible for the increase in flow and that local reduction of tissue $PO_2$ which accompanies increased metabolic activity contributed in part to this process. Conversely, it was demonstrated that CBF decreased during anesthesia and during states of reduced metabolism when tissue $PCO_2$ production and oxygen utilization was reduced.

(i) Carbon Dioxide

Since carbon dioxide is the main active product of cerebral metabolism, it provides a mechanism of regulating local blood flow in accordance with the metabolic needs of the tissue. Carbon dioxide has been found to have the most profound effect on cerebrovascular tone of any substance investigated. Inhalation of 5 percent $CO_2$ increases CBF by approximately 50 percent while 7 percent raises CBF by more than 100 percent (39,40). Measurements of the effect of higher concentrations of $CO_2$ in man have not been reported, however , an increase in CBF of nearly 240 percent of normal at arterial $CO_2$ tensions ($PaCO_2$) of 150 mm Hg have been reported in monkeys (41). Harper (42) showed that CBF increased by 100 percent on raising the $PaCO_2$ from 40 mm Hg to 80 mm Hg in dogs. The $CO_2$ effect was attenuated by rendering the animals hypotensive, suggesting that cerebral vasodilation was partially due to hypotension.

Reducing arterial $CO_2$ tension by hyperventilation causes an increase in cerebrovascular resistance and a decrease in cerebral blood flow. Kety (43) demonstrated that a reduction of arterial $CO_2$ to 26 mm Hg in man caused a decrease in CBF by 40 percent, whereas Reivich (44) demonstrated a fall in CBF of 50 percent when $PaCO_2$ was lowered to 19 mm Hg. Alexander, _et al_. (45) reduced $PaCO_2$ in man under nitrous oxide anesthesia to 11 mm Hg and found that jugular venous $PO_2$ levelled off at a value near 19 mm Hg as the $PaCO_2$ was reduced to 20 mm Hg or lower. The authors suggested that a maximal vasoconstriction is produced at values of $PaCO_2$ below 20 mm Hg and that vasodilator effects of low tissue oxygen counteracted any further vasconstriction from a further lowering of $PaCO_2$.

The changes in $PaCO_2$ studied in man, although producing marked alterations cerebrovascular resistance, are not associated with any significant changes in cerebral oxygen uptake. At $PaCO_2$ levels below 20 mm Hg, however, significant changes in cerebral carbohydrate metabolism have been demonstrated (45). The factor responsible for this shift towards anaerobiosis during extreme hypocapnia has been shown by Reivich (46) to be due to cerebral hypoxia and not to a specific effect of hypocarbia.

Although the response of cerebral vessels to changes in $PaCO_2$, $PaO_2$ and pH have been well documented, the mechanisms and the site of action remain to be fully elucidated. Severinghaus (47) and Skinhoj (48) have maintained that the pH of the CSF or interstitial fluid is the important factor governing the regulation of cerebrovascular tone and cerebral blood flow. Gotoh, et al. (21) have presented evidence to support the hypothesis that the intracellular pH of the smooth muscle cells of cerebral arterioles is responsible for cerebral vasomotor activity. The latter concept has been recently confirmed by Shinohara (49) who demonstrated that the final common pathway, responsible for cerebral vasomotor activity was a change in the intracellular hydrogen ion concentration of smooth muscle fibers of cerebral arterioles. The precise mechanism whereby hydrogen ion concentration controls vasomotion is not known, but is effect on intracellular calcium ion concentration appears to be important.

(ii) Oxygen

There is conflicting evidence regarding the effect of elevated arterial tensions of oxygen on the cerebral circulation. Inhalation of 85-100 percent oxygen has been reported to produce a mild reduction in cerebral blood flow in the order of 12-15 percent (50,51), while other investigators (52,53) demonstrated essentially no change in CBF when 80 percent oxygen was inhaled with $PaCO_2$ kept constant. However, during extreme hypocapnic states ($PaCO_2$ 20 mm Hg or less) a vasoconstrictor effect and a further reduction of CBF of approximately 25 percent has been demonstrated by inhalation of oxygen at 2-3.5 atomospheres (46). Two theories have been advanced to explain the vasoconstriction effect present under these conditions. First, hyperbaric oxygen has a direct vasoconstrictor effect, and second that by removing the vasodilator effect of hypoxia associated with marked hypocapnia, the hyperbaric oxygen allows the full vasoconstriction effect of low $PaCO_2$ to be demonstrated.

Numerous studies have demonstrated the effect of decreased arterial oxygen tension on cerebral perfusion (51,52). Inhalation of 8-10 percent oxygen increased cerebral blood flow by approximately 35 percent with the $PaCO_2$ remaining constant. A fairly high threshold for the response of cerebral blood flow to hypoxia appears to be present. No significant increase in CBF was observed until the oxygen tension of the blood is decreased to below 50 mm Hg (14,41,54). Harper (14) found no change in flow through the cerebral cortex until the $PaO_2$ fell below 40 mm Hg. Thereafter, cerebral blood flow increased as the $PaO_2$ fell, and at a $PaO_2$ level of 20 mm Hg, the CBF was twice that of the control value.

The mechanisms whereby oxygen affects the tone of the cerebral vessels has not been clarified. Gotoh and Meyer (55) have postulated that oxygen directly interacts with specific chemoreceptors within the vessels walls, although they have not excluded a neural mechanism.

In summary, recent studies have shown that the relative effectiveness of chemical control (carbon dioxide, oxygen and other metabolites) in regulating cerebral blood flow varies with the tension of the gas being considered. Within the normal range, arterial $PCO_2$ is predominant in regulating CBF while arterial $PO_2$ has an insignficant effect. In this range the oxygen-dissociation curve is relatively flat so that large changes in $PaO_2$ produce little change in arterial oxygen content or cerebral oxygen delivery. The sensitivity of cerebral vessels to changes in arterial $PCO_2$ is markedly diminished at tensions above approximately 80 mm Hg. On the other hand, oxygen tensions of 1 atmosphere or more appear to produce moderate reductions in CBF. At $PaO_2$ levels below 50 mm Hg, the effect of hypoxia in raising CBF becomes more important and more pronaunced in spite of a concomitant hypocapnia which is usually present. In fact the limiting factor in the reduction of CBF by a low $PaCO_2$ appears to be the development of cerebral hypoxia. Thus, at critically low levels of oxygen tension, the effect of oxygen becomes more predominant and that of carbon dioxide negligible. Cerebral blood flow therefore, appears to be normally regulated in response to the metabolic requirements of the brain by carbon dioxide, while the effect of oxygen becomes important only after the brain $PO_2$ becomes critically low or markedly elevated.

(b) Neurogenic Control of Cerebral Blood Flow

The existence of nerve fibers on cerebral vessels has been thoroughly documented by gross observations since the seventeenth century. Classical light microscopic (56,57,58), fluorescent histochemistry (27,28,29,59) and electron microscopic studies (30,31,60 61) have all contributed to the morphologic evidence for cerebral vascular innervation. However, there remains considerable uncertainty as to the functional significance of these autonomic fibers.

The application of fluorescent microscopy with its specific identification of catecholamine-containing nerves and electron microscopy with its high resolution and potential for differentiating between afferent and efferent endings has renewed interest in this problem. Utilizing the histochemical fluorescence technique, Hillarp (62) and Falck, et al. (27), and Nielsen (28) demonstrated a rich adrenergic nerve supply to the main arteries at the base of the brain. Fewer nerves were present in distal branches, but pial arteries as small as 15-20 microns in diameter possessed adrenergic nerves. These nerves were infrequently observed in the arteries penetrating the brain parenchyma. Evidence that these nerves contained noradrenaline was given by their increased fluorescence after injection of a monoamine oxidase inhibitor and complete disappearance of fluorescence after reserpine treatment. In support of a sympathetic origin was the total loss of fluorescence of all vascular nerves following bilateral cervical sympathectomy.

Peerless and Yasargil (29) also found a rich adrenergic innervation of superficial cerebral vessels with diameters between

100-300 microns. Fluorescent nerves were followed in diminishing numbers on all superficial arteries down to a diameter of approximately 15 microns while intracerebral vessels were sparsely innervated. When the animals were subjected to drug-induced extreme hypertension and hypotension, a marked decrease of noradrenaline content in the nerve terminals resulted.

Recent electron microscopic studies (30,31) have demonstrated the presence of myelinated and unmyelinated nerves within the adventitia or at the adventitial-medial junction of the larger cerebral vessels in various species, including man. The closest approximation observed between axonal endings and smooth muscle plasmalemma was 800 angstroms. The terminals of these nerves contained granular and agranular vesicles and it was proposed that the nerves containing granular vesicles were sympathetic (noradrenaline containing) in origin and are concerned with a vasoconstriction function, whereas axons containing translucent vesicles (acetylcholine containing) were of parasympathetic origin and are concerned with a vasodilatory mechanism.

Despite the morphological demonstration of an abundant innervation, the functional role of these nerves remains to be fully elucidated. Recent physiological studies have contributed to a better understanding of neurogenic mechanisms involved in the control of cerebral vasomotor tone and cerebral blood flow.

### (i) Sympatheteic Control of Cerebral Blood Flow

Krog (26) demonstrated a reduction in internal carotid artery flow in man on stimulation of the cervical sympathetic chain and Meyer, et al. (63) made similar observations in monkeys. In Meyer's study reduction in internal carotid artery flow was approximately 25 percent and the reduction was much greater during inhalation of 5 percent carbon dioxide. James (25) meausred cerebral blood flow in baboons during sympathetic stimulation and found that stimulation attenuated and sympathectomy accentuated the dilator effects of inspired carbon dioxide. These studies have been confirmed by Harper (24). Correlating morphological and physiological findings from various recent studies, Harper, et al (24) proposed that the cerebral circulation operated as two resistance units in series, each under a different control system: (i) intraparenchymal resistance -- includes intraparenchymal arterioles and these vessels are regulated locally by the products of cerebral cellular metabolism and by changes in blood gases and (ii) extraparenchymal resistance -- which includes extraparenchymal arteries and veins under autonomic nervous control. These investigators speculated that although the extraparenchymal vessels are influenced by sympathetic discharge and may constrict the inflow vessels, normal autoregulatory process affecting the intraparenchymal arterioles will maintain normal cerebral blood flow. Furthermore, it was speculated, that during hypercapnic states the intraparenchymal system is paralyzed and thus the effects of sympathetic influence are uncovered.

### (ii) Parasympathetic Control of Cerebral Blood Flow

Chorobski and Penfield (64) first described vasodilator fibers in facial nerve passing through the geniculate ganglion via the greater superficial petrosal nerve to internal carotid plexus and then to the intracerebral vessels. Forbes, et al. (13) demonstrated vasodilation of the cerebral vessels with a concomitant increase in cerebral blood flow after stimulation of the vagus and facial nerves.

Futhermore, the importance of cholinergic nerves in cerebral vasodilation has been demonstrated in the studies of Mchedleshvili (65) and Salanga (66). However, Meyer, et al. (34) reported that increases in CBF induced by stimulation of the trigeminal, facial, glossopharyngeal and vagus nerves were inconsistent and unreliable and when they occurred, could be accounted for by increases in cerebral metabolism.

(iii) Central Neurogenic Control of Cerebral Blood Flow

Molnar (67) electrically stimulated areas of the brain stem in cats and from these studies concluded that neurogenically mediated centers exist in the brain stem which control vasoconstriction and vasodilation. Langfitt, et al. (68) demonstrated an increase in internal carotid flow of monkeys by 40 percent after electrical stimulation of the brain stem. Shalit (69) studied the response of the cerebral vasculature to carbon dioxide after inducing freezing lesions within the brain stem and postulated the existence of neuromechanisms in the pons which respond to carbon dioxide causing vasodilation. More recently Fujishima (70) noted decreased CBF and cerebral metabolism in the dog following basilar artery occlusion along with reduced responsiveness of cerebral vessels to increased carbon dioxide tension.

Meyer et al. (34) measured CBF and cerebral metabolic rate of oxygen ($CMRO_2$) during electrical stimulation of the cortex, brain stem areas and diencephalon in monkeys and demonstrated that cortical stimulation had no effect on CBF and metabolism whereas stimulation of the thalamus, hypothalamus, pontine and midbrain retuclar formation increased cerebral blood flow by approximately 40 percent. Stoica, et al. (71) stimulated brain stem structures in baboons with pharmacologic agents and produced large and signficant increase in cerebral blood flow. In this study evidence was presented to support the postulate that vasoconstrictor (adrenergic) and vasodilator (cholenergic) mechanisms exist within the brainstem which control the tonus of the large cerebral vessels. These investigators proposed (in accordance with Harper's hypothesis) that the central neurogenic vasomotor mechanisms exert their main influence via the large extraparenchymal vessels and that they, as well as the intraparenchymal vessels, are influenced by changes in arterial carbon dioxide tensions.

From the studies outlined above, it becomes apparent that centers exist within the brain which influence CBF and cerebral metabolism. The fact that these centers correlate closely with the central pathway of the sympathetic and parasymathetic systems suggests that control of CBF in part may be mediated via this neurogenic pathway. Furthermore, clinical and experimental studies have demonstrated that the mechanism of autoregulation requires the integrity of both the brain stem centers and the autonomic nervous system. (72).

## II. MEASUREMENT OF CEREBRAL BLOOD FLOW

### A. The Inert Tracer Techniques

The year 1945 is important in the history of research on the cerebral circulation for it was during this year Kety and Schmidt(73) developed the nitrous oxide method for quantitatively measuring cerebral blood flow and cerebral oxygen utilization in man. This technique, based on the Fick Principle (1855) has been widely adapted and modified in recent years. Several of the more common methods for calculation of the average of regional cerebral blood flow based on the

Fick Principle are outlined below.

I. Arterio-Venous Difference Techniques:
  (i) $N_2O$ - Manometric Counting
  (ii) $^{85}Kr$ - Scintillation Counting
  (iii) Argon - Mass Spectometry

II. Isotope Clearance (Residue Detection) Techniques:
  (i) $^{133}Xe$ - Scintillation Counting
  (ii) $^{85}Kr$ - Scintillation Counting
  (iii) $^{15}O$ - Scintillation Counting.

The inert gas techniques utilize the Fick Principle which states that the quantity of inert gas taken up by a tissue in a given time is equal to the quantity entering the tissue via the arterial blood, minus the quantity leaving the tissue in the venous blood. Expressed mathematically:

$$CBF = \frac{\lambda C_v(u)}{\int_0^u (C_a - C_u)dt}$$

where $C_a$ and $C_v$ equal the cerebral arterial and venous concentrations of the inert gas respectively, $\lambda$ is the partition coefficient for the inert gas between brain tissue and blood, and $C_v(u)$ is the venous concentration of the inert gas at time u, i.e., when the brain concentration of the inert gas is in equilibrium with the venous blood at the end of the period of inhalation.

Thus, by determining the difference between the integrated values of the concentration of the inert gas in arterial and venous blood of the brain, and knowing the value of the gas in the venous blood at time $u_1$, the average or total blood flow per unit volume of brain was calculated (Kety, 1945).

The nitrous oxide method of Kety and Schmidt determined the average cerebral blood flow per 100 grams of tissue per minute. Several assumptions regarding calculations of CBF were made by the authors: (i) CBF was constant during the period of measurement and was not altered by inhalation of the inert gas; (ii) the venous samples were representative of mixed venous blood from the brain: (iii) there was an insignificant amount of extracerebral contamination; (iv) blood samples are drawn over a sufficient long period so that the concentration of the inert gas in the brain is in equilibrium with the concentration in the venous blood from the brain; and (v) the partition coefficient value used was representative of the mean partition coefficient of the inert gas for the entire brain.

Recent studies by Lassen (74), Mangold (75), Reivich (41), Shenkin (76) and Kety (43,51,73) have demonstrated that the assumptions upon which the inert gas techniques are based appear to valid and measurement of CBF under normal physiological conditions can be made.

Several modifications of the original Kety-Schmidt technique have been developed for measurement of the CBF. Scheinberg (77) described a method of CBF measurement in which an integrated sampling technique was utilized, and Kennedy (78) measured CBF in children by a microanalytical method for determining the concentration of nitrous oxide.

In 1951 Kety (79) proposed that cerebral blood flow could be determined by a desaturation technique and suggested the use of radioisotopes. Lassen and Munck (74) introduced $^{85}$Krypton for cerebral blood flow measurement utilizing the Kety-Schmidt method and Lewis (80) devised a technique whereby estimation of total CBF was recorded by means of external counting of $^{79}$Krypton with externally placed scintillation detectors.

While the Kety-Schmidt nitrous oxide technique or its modifications were suitable for studies of physiological and pathological changes which affected the whole brain (general anesthesia, coma, dementia, epilepsy and other diffuse brain disorders), this method of CBF measurement has been of limited clinical and experimental value since most of the acute brain disorders are of a localized nature (apoplexy, tumors, trauma). This fact prompted endeavours to develop techniques which were capable of measuring regional cerebral blood flow.

In their classical experimental studies, Lassen and Ingvar (81, 82) developed the radioisotope clearance method for the quantitatvie measurement of regional cerebral blood flow. In their initial experiments, rCBF was determined by recording the rate of clearance of $^{85}$Krypton from the exposed cerebral cortex following internal carotid artery injection using a beta ray ($\beta^-$) detector. This technique was subsequently adapted for measurement of rCBF in man by recording the clearance of gamma emissions of $^{85}$Krypton through the intact skull(83). Glass and Harper (84) measured rCBF in man by substituting $^{133}$Xenon for $^{85}$Krypton.

The intra-arterial injection isotope clearance (residue detection) methods for studying cerebral blood flow have become very popular in recent years and arrays of up to 250 scintillation detectors have been utilized to determine clearance rates from smaller regions of the brain (80). This technique is currently advocated as the most physiological means to measure rCBF and has assumed increasing clinical importance as an adjunct to cerebral angiography.

A radioactive oxygen method has been recently developed by Ter-Pogossian and his associated (85). This technique utilizes $^{15}$Oxygen either as oxyhemoglobin or water injected into the internal carotid artery with the clearance curves determined by external scintillation detectors. This method has an advantage over the $^{133}$Xenon method in that both rCBF and regional cerebral metabolism are measured simultaneously. Theoretically $^{15}$Oxygen also offers other advantages over $^{133}$Xenon in measurement of rCBF in that the problems of self-absorption are less ($\gamma$-ray energy consideration) and the partition coefficients in both cerebral compartments are equal. Disadvantages of the $^{15}$Oxygen technique include difficulties with $\gamma$-ray collimation and a very short half life (123 seconds) of the radioisotope.

In general, three methods of analysis and calculation of the cerebral blood flow have been utilized. These methods include (i) Bi-exponential (Compartmental), (ii) Stochastic (Height over Area), and (iii) Initial-slope-index (ISI) methods. Theoretical considerations and limitations of each method are discussed in section B.

Mallett and Veall (86) introduced an atraumatic technique for the measurement of rCBF by monitoring the clearance of radioisotope from the brain after five minutes of inhalation of $^{133}$Xe mixed with air. Although very attractive clinically, the technique has been associated with the problem of recirculation of the radioisotope and extracerebral contamination. Obrist, et al . (87) refined the $^{133}$Xe inhalation technique by utilizing a three-compartment analysis of the cerebral clearance curves. In addition, a deconvolution procedure (unfolding of superimposed responses) was utilized to correct for recirculation. More recently Obrist (88) further modified the analysis of the inhalation technique and obtained grey matter flow values comparable to those of the intra-arterial technique.

Agnoli, et al. (89) proposed employing an intravenous $^{133}$Xe method for measuring rCBF. By solving the convolution integral for each compartment of the clearance curve separately using an analog computer, Austin, et al. (90) measured rCBF in the grey and white matter after intravenous injection of $^{133}$Xe. The flow values obtained by this method compared favorably with those determined by the intra-arterial technique. Because of its relative atraumatic nature, the intravenous method should gain wide application in clinical studies.

B. Theoretical Considerations of CBF Calculation

(a) Bi-exponential (Compartmental) Analysis

Kety (73) showed that the exchange of an inert gas between blood and a single homogenous tissue $i$ could be expressed by application of the principle of conservation of matter (i.e., the Fick Principle, when applied to blood flow measurement). Expressed algebraically:

$$dQ_i = F_i dt(Ca - Cv_i) \qquad \text{-B1}$$

Here, $dQ_i$ is the infinestimal change in the quantity of inert gas in the tissue in time dt, and includes, by definition, its contained blood, which, by itself, constitutes only a small fraction of the total tissue weight $W_i$. Ca and $Cv_i$ are respectively, the arterial and outflow venous concentrations of inert gas for tissue $i$. In the following, the subscript $i$ is dropped for the sake of clarity whenever a single tissue is under consideration.

In a single homogeneous tissue with an essentially instantaneous diffusion equilibrium, the tissue concentration C is given by

$$C = \lambda\ Cv \qquad \text{-B2}$$

where λ is the tissue-blood partition coefficient as defined by Kety (1951). However, since the total quantity of inert gas, Q, is given by

$$Q = CW,$$

the following expression for dQ becomes;

$$dQ = WdC \qquad \text{-B3}$$

since W, the total weight, stays constant. Substituting for Cv (from equation B2)and for dQ (from equation B3) into equation B1 yields:

$$WdC = F\ (Ca - \frac{C}{\lambda})dt$$

or

$$\frac{dC}{dt} = - \frac{F}{W\lambda}(C(t) - Ca\lambda) \qquad \text{-B4}$$

Here, $\frac{F}{W}$ = f, the blood flow per gram of tissue. If Ca=0, and if, at t=0 (the start of inert gas clearance from the tissue), C(t)=0, equation B4 may be integrated to give

$$C(t) = C(0)\exp[\frac{-f}{\lambda}\ t] \qquad \text{-B5}$$

where C(t) : Tissue concentration at arbitrary time t;

C(0) : Concentration at t=0, or the peak value prior to desaturation;

f : Blood flow in units of ml/gm/min;

λ : The volume distribution of the tracer per unit weight of tissue in units of ml/gm.

Equation B5 therefore describes the behavior of the concentration as a function of time, and clearly shows a monoexponential clearance.

Subsequent to this development of Kety (1951), Ingvar and Larsen (82) recognized that clearance curves from the brain could be appropriately described by a model consisting of two physiologic compartments in parallel. This became apparent upon replotting such curves on a semi-logarithmic scale, whereby it became fairly obvious that two distinct rates of decay were present.

Ingvar and Lassen showed that in a inhomogeneous organ (such as the brain), the average concentration $\bar{C}(t)$ is given by the following equation:

$$\bar{C}(t) = \frac{1}{\sum_i W_i} \sum [W_i C_i(0) \exp(\frac{-f_i}{\lambda_i} t)] \qquad \text{-B6}$$

The summations in equation B6 run over all possible values of the suffix $i$, which labels the different compartments; i.e., in a two compartment model, $i$ would take two values. The average flow through the organ, $\bar{f}$, is

$$\bar{f} = \frac{1}{\sum_i W_i} \sum f_i W_i \qquad \text{-B7}$$

If one assumed rapid arrival of the tracer bolus into the tissue, even mixing of the tracer, and uniform counting efficiency, then, for each compartment $i$, $C_i(0)$ is directly proportional to $f_i$, i.e.,

$$C_i(0) = Df_i,$$

D being a relative measure of the quantity of tracer injected.

For a two-compartment system, the average concentration becomes, from equation B6,

$$\bar{C}(t) = W_g f_g D \exp(\frac{-f_g}{\lambda} t) + W_w f_w D \exp(\frac{-f_w}{\lambda} t) \qquad \text{-B8}$$

The suffixes $g$ and $w$ in equation B8 label the two compartments, and will, in the case of the brain, be subsequently interpreted to mean grey and white matter respectively. In addition, the quantities $W_g$ and

$W_w$ are relative weights of the two tissues, i.e., $\Sigma W_i$ in equation B6 becomes $W_g + W_w = 1$.

Defining the four quantities $I_g$, $I_w$ $K_g$ and $K_w$ as follows:

$$I_g = W_g f_g D \quad ; \quad K_g = \frac{fg}{\lambda g}$$
$$I = W_w f_w D \quad ; \quad K_w = \frac{fw}{\lambda w} \qquad \text{-B9}$$

one rewrites equation B8 as follows:

$$\bar{C}\,(t) - I_g \exp\,(-K_g t) + I_w \exp\,(-K_w t)$$

If the partition coefficients $\lambda_g$, $\lambda_w$ are known, then the flow rates $f_g$ and $f_w$ may be calculated by replotting the clearance curve on a semilogarithmic scale. This replotting gives the two components (as straight lines), and their respective "half time" (t½'s). The flow rates are related to the t½'s via:

$$f_g = \lambda_g \frac{0.693}{t½_g} = 100\lambda_g \frac{0.693}{t½_g} \text{ ml/100/gm/min} \qquad \text{-B10}$$

$$f_w = \lambda_w \frac{0.693}{t½w} = 100\ {}_w \frac{0.693}{t½w} \text{ ml/100/gm/min} \qquad \text{-B11}$$

The $t½_w$ and $t½_g$ are the respective "half-times" for the two compartments. From the zero-time intercepts, $I_g$ and $I_w$ (as given by equation B9), one obtains the relative weights of the tissues, via.:

$$W_g = 100 \frac{I_g/f_g}{I_g/f_g + I_w/f_w}\% \qquad \text{-B12}$$

$$W_w = 100 \frac{I_w/f_w}{I_g/f_g + I_w/f_w}\% = 100(1-W_g)\% \qquad \text{-B13}$$

Then the mean blood flow for the two compartments (as defined by equation B7 becomes:

$$\bar{f}_C = f_g W_g + f_w W_w \text{ ml/100gm/min} \qquad \text{-B14}$$

(b) Stochastic (Height/Area) Analysis:

The basic equation in this approach to CBF was derived by Meier and Zierler (91) and is based on measurement of indicator transit times. It is, in fact, derived from the principle of conservation of matter (Fick Principle) which states that all of the indicator introduced into the system must, in time, be washed out. Based on this, one derives an expression from the mean transit time ($\bar{t}$) of the injected indicator which is

$$\bar{t} = \frac{V}{F} \text{ min.} \qquad \text{-B15}$$

In equation B15, F is the flow through the system. V is the equilibrium volume of distribution of the indicator, and is that imaginary volume of blood which, at equilibrium, contains the same amount of indicator as the whole brain. It is, therefore, neither the total volume of the brain nor its blood volume. Thus,

$$V = \frac{\text{Quantity of tracer in the whole blood at complete equilibrium}}{\text{Concentration of tracer in the blood at equilibrium}} \qquad \text{-B16}$$

Since inert diffusible tracers dissolve physically in all the various tissue components, having approximately the same solubility in 1 gram of brain as in 1 ml of blood, it follows that

(i) V is of the same order of magnitude as the brain volume, and therefore much larger than the cerebral blood volume; and

(ii) Variation in the blood volume within the brain does not significantly change V.

At equilibrium, inert gases are distributed passively according to solubility properties only. This means that the ratio

$$\frac{\text{Quantity of tracer in 1 gm of brain at complete equilibrium}}{\text{Quantity of tracer in 1 ml of blood at complete equilibrium}} = \lambda \frac{\text{ml}}{\text{gm}} \qquad \text{-B17}$$

defines the partition coefficient λ, and is practically a constant, with a value close to 1.0.

From the definitions of V and λ (as given by equations B16 and B17), it follows, therefore that for a brain of weight W gm,

$$\lambda = \frac{V}{W}\ \frac{\text{ml}}{\text{gm}} \qquad \text{-B18}$$

i.e., the solubility ratio between brain and blood (partition coefficient) equals the volume of distribution of the tracer per gram of tissue.

Zierler (92) applied the stochastic theory to the problem of measurment of CBF by external monitoring of a radioisotope and showed that the mean transit time $\bar{t}$ of the indicator through the brain tissue could be written as the ratio of the area under the clearance curve, A, to the initial height H

$$\bar{t} = \frac{A}{H}\ \text{min} \qquad \text{-B19}$$

This relationship is arrived at by considering the following: When an indicator is rapidly injected (spike, impulse, or delta funtion injection) into the arterial inflow of the brain, the detector will initially detect, within its field of view, all the indicator particles which are to be cleared by the tissue. The initial height of the resulting washout curve, H, corresponds to the total number of particles, and the area A of the ensuing clearance curve corresponds to the sum of all the transit times of the particles.

The mean transit time represents the weighted sum of all the transit times (using as the weighting factor the frequency with which the individual transit times occur), i.e.,

$$\bar{t} = \frac{\int_0^\infty Q(t)dt}{Q_{max}}$$

where Q(t) is the quantity of tracer remaining at time t, and $Q_{max}$ is the total amount of tracer injected (i.e., the peak value).

$$\bar{t} = \frac{\text{Area under washout curve}}{\text{Height of the curve}}$$

$$= \frac{\text{Area of externally recorded curve after bolus injection}}{\text{Height of externally recorded curve after bolus injection}}$$

Equation B15 is rewritten as follows:

$$\bar{t} = \frac{V}{F}$$

$$= \frac{V/W}{F/W} \qquad \text{-B20}$$

Using the definition of $\lambda$ (as given by equation B18) and defining f as the blood flow per gram of tissue, i.e.,

$$f = \frac{F}{V},$$

equation B20 becomes

$$\bar{t} = \frac{\lambda}{f} \qquad \text{-B21}$$

Combining the two definitions of $\bar{t}$, i.e., equations B19 and B21, one gets that

$$\frac{A}{H} = \frac{\lambda}{f},$$

or

$$f = \frac{\lambda H}{A}\ \text{ml/gm/min}$$

In terms of conventional units expressing rCBF as the flow through 100 gms of tissue per minute, this becomes

$$\text{rCBF} = 100\lambda\frac{H}{A}\ \text{ml/100/gm/min} \qquad \text{-B22}$$

This equation enables a quantitative measure of blood flow per unit weight (100gm) to be obtained without it being necessary to estimate the weight of the brain, and having only information regarding the volume of distribution per gm of tissue, i.e., $\lambda$.

The principal difficulty in employing equation B22 for CBF measurment lies in the determination of the area under the curve, which necessitates either collection of data up to infinite times (which is impossible in practice), or an extrapolation of the data beyond the finite time of observation. This extrapolation has been performed by plotting the clearance curve on semi-logarithmic paper and estimating the "half-time" of the "tail" portion of the curve. Then the extrapolated area from 10 (or 15) minutes to infinity is given by the product of the height of the curve at 10 (or 15) minutes and the quantity $\frac{t_{½}\ \text{tail}}{0.693}$.

Without utilizing such an extrapolation procedure, a 10 minute washout curve yields values of CBF which are in moderate overestimation (approximately 10%) of the true CBF.

(c) Initial-Slope-Index Analysis

An approximation of CBF may be derived from the logarithmically recorded initial phase of the $^{133}$Xe clearance curve (93,94). The basic premise underlying the initial-slope-index of CBF measurement is the observation that the grey substance dominates the initial portion of the clearance curve. This dominance is so marked in the first one or two minutes that the washout of tracer can be expressed, at least in this period of time, as a monoexponential function.

The monoexponential clearance curve of an inert gas from a tissue is described by equation B5:

$$C(t) = C(0)\ \exp\left(\frac{-f}{\lambda}\ t\right) \qquad \text{-B23}$$

Once again, in equation B23, C(t) is the concentration of the tracer in the tissue at time t, C(0) is the initial concentration (maximum height of the clearance curve after a delta function injection); f represents the blood flow (in ml/gm/min) λ, the tissue:blood partition coefficient (in ml/gm); and t, the time (in min).

Taking the logarithm (base e) of equation B23 and then differentiating with respect to time t, one gets

$$\frac{d}{dt} \ln C(t) = -\frac{f}{\lambda}$$

i.e.,

$$f = -\lambda \frac{d}{dt} \ln C(t)$$

$$= \lambda\alpha \text{ ml/gm/min} \qquad \text{-B24}$$

where $\alpha$ is the numerical value of the slope of the logarithmically recorded clearance curve. Converting from natural logarithms (base e) to logarithms (base 10), equation B24 becomes

$$f = -\lambda \ln 10 \cdot \frac{d}{dt} \log C(t)$$

$$= D\lambda \ln 10 \cdot \text{ml/gm/min}$$

D is the numerical value of the slope in the logarithm (base 10) scale, and $\ln 10 = 2.30$ converts natural to base 10 logarithm.

Thus, an estimate of CBF is given by

$$f_{ISI} = 2.30 \lambda_g D_{initial} \text{ ml/gm/min} \qquad \text{-B25}$$

$\lambda_g$ being the partition coefficient of the $^{133}Xe$ between blood and the grey matter of the brain. Once again, in conventional terms, rCBF is given by

$$rCBF_{ISI} = 100\lambda_g \cdot 2.30\, D_{initial} \text{ ml/100 gm/min} \qquad \text{-B26}$$

## III. STUDIES ON CEREBRAL VASOSPASM

Although it had been hypothesized that cerebral vasospasm was a causative factor in the pathogenesis of disorders such as epilepsy, migraine, transient hemiplegia, aphasias and other temporary neurological phenomena as early as the beginning of the last century, research on the reactivity of the cerebral vessels was not performed until 1866. At this time Schultz (95) demonstrated vasoconstriction in pial vessels on electrical stimulation. Apart from this work, little interest was taken in this field until Florey (1925) reported pial vessel spasm in cats by direct mechanical and electrical stimulation. Florey's work was reproduced and confirmed with photography by Riser and his associates in 1931.

Echlin (96) was the first investigator to systematically study the reactivity of the superficial arteries of the brain. He noted that the larger arteries were more refractive to mechanical and electrical stimulation and a definite species difference in the reactivity of the pial vessels was present. More important, Echlin demonstrated using thermoelectric blood flow recordings and intravital staining techniques that vasoconstriction produced ischemia in the cortical areas distal to the induced vasospasm. This vasospasm was refractory to topical application of acetylcholine and amyl nitrate and to inhalation of carbon dioxide. Echlin, like Florey, proposed that induced vasospasm was independent of a neurogenic mechanism.

Lende (97) studied cerebral vasospasm induced by mechanical and electrical stimuli and found a gradient in vessel reactivity with spasm being more pronounced in the larger arteries. He postulated that spasm was not mediated by autonomic fibers since the response was unaffected by sympathectomy or nerve blockade. Several pharmacologic agents were topically applied to spastic vessels, and of the various drugs listed, Phentolamine was found to be most effective in preventing the induction and in relieving spasm. Carbon dioxide failed to prevent induction of cerebral vasospasm.

Further observations that the larger vessels of the circle of Willis in cats, dogs and monkeys contract vigorously on mechanical stimulation have been reported by Pool (98), Raynor (99) and Corday (100). Vasospasm of the larger branches of the circle of Willis in humans has been frequently observed after mechanical stimulation during cerebral operations (58,98,101,102,103). However, an important and apparently universal observation regarding vasospasm after mechanical stimulation has been its relatively short duration (rarely beyond 30 minutes).

Since the advent of clinical cerebral angiographical studies the literature on cerebral vasospasm following subarachnoid hemorrhage has increased at a remarkable rate but the basic etiology remains in doubt. Factors which appear to be of primary importance in the pathogenesis of cerebral vasospasm after aneurysmal rupture are generally classified into mechanical, chemical and neurogenic mechanisms.

### A. Mechanical Factors

Ecker and Riesmenschneider (104) in 1951 demonstrated with angiography evidence of cerebral vasospasm in 10 patients with recent subarachnoid hemorrhage and concluded that the common element in the production of pathological vasoconstriction appeared to be abrupt traction on the arterial wall. Johnson, *et al*. (103) offered evidence that spasm was produced and maintained by the mechanical effects of the subarachnoid hemorrhage. They suggested that after an aneurymsal rupture, subarachnoid blood would surround and throw the susceptible arteries into spasm by physcial stretch or by pull on attached arachnoidal bands. Maintenance of the spasm was effected through intrinsic damage to the vessel wall or by a surrounding blood clot. Further support for the mechanical hypothesis with respect to the genesis of vasospasm came from the studies of Suwanwela and Suswanwela (105) in patients with head injuries. These authors noted that angiographical spasm of the major arteries at the base of the brain was present in only 5 percent of patients. Since the majority of patients with traumatic subarachnoid hemorrhage showed no evidence of arterial spasm, they suggested that a mechanical stimulus rather than blood in the cerebrospinal fluid was responsible for cerebral arterial spasm.

Echlin (106) suggested that traumatic rupture of the vessel wall itself may be an important factor in the genesis of vasospasm. He proposed that vasospasm could be due to mechanical irritation or sensitization of the vessels after aneurysmal rupture but conceded that prolonged vasomotor reflex spasm by vessel injury has not been demonstrated.

Recent studies by Arutuinov, et al. (107) have demonstrated that cerebral arteries are stabilized within the subarachnoid spaces by fibrous structures they termed "chordae." These chordae are surrounded by nerve fibers, receptors and pseudounipolar nerve cells and are intimately related to nerve elements of the arterial adventitia (perivascular nerves). These investigators demonstrated intense short-term spasm of the basilar artery in cats after mechanical irritation of the surrounding chordae. They proposed that the hemodynamic impact of the blood jet streaming from a ruptured aneurysm could stimulate chordal and perivascular nerves, causing the development of short-term vasospasm. However, from these studies the authors concluded that mechanical factors did not contribute to the pathogenesis of chronic cerebral vasospasm.

## B. Chemical Factors

### (a) Serotonin Studies

Because cerebral vasospasm is frequently associated with subarachnoid hemorrhage after aneurysmal rupture and appears to be slowly alleviated as the extravasated blood is absorbed, Raynor, et al. (99) proposed that some substance in the blood was the etiologic factor in the genesis of cerebral vasospasm. Serotonin (5-hydroxytryptamine), a normal constituent of blood platelets, was selected for study because of its marked constrictor action on peripheral arteries. Previously, Zucker and Borrelli (108) had demonstrated that half of the platelet serotonin was released from platelets during coagulation and the remainder became available during the next several days. Raynor, et al. (109) demonstrated an intense and prolonged spasm of the larger arteries in cats after topical application of serotonin. From this study they postulated that after a subarachnoid hemorrhage, serotonin was released from disrupted platelets during the process of coagulation and caused prolonged vasospasm. Support for this hypothesis came from Buckell(110) who demonstrated elevated levels of serotonin in the hematoma fluid surrounding ruptured intracranial aneurysms that were associated with arterial spasm in three patients. Karlsberg, et al. (111) produced marked cerebral vasoconstriction in monkeys with intracarotid infusion of serotonin with dosages as low as 10 mg/kg/minute for 5 minutes. The vasoconstrictor response to serotonin infusion was abolished by prior intracarotid administration of methysergide (a potent serotonin antagonist). Furthermore, methysergide partially reversed the serotonin induced vasoconstrictor effect when infused in high concentration. Brawley, et al.(112) consistently produced vasoconstriction of the internal carotid artery in dogs after injection of serotonin into the carotid circulation. Pre-treatment of the animals with methysergide completely blocked the arterial response to serotonin. In addition, when methysergide was administered after serotonin, the induced vasoconstriction was reduced but not completely ameliorated. However, methysergide did not appear to produce a beneficial effect on induced chronic vasospasm and it was proposed that serotonin was probably not the etiologic agent producing chronic cerebral vasospasm.

Echlin (113) was first to demonstrate that the application of autogenous blood against the walls of large cerebral arteries produced intense vasoconstriction. This phenomenon has been confirmed in dogs, cats, rabbits and monkeys. Echlin concluded that, although topical application of serotonin frequently resulted in intense vasospasm, it was less marked than that caused by application of fresh autogenous blood. Kapp, el al. (114) studied various fractions of blood and reported the isolation of a vasoactive substance from feline platelets which produced marked constriction of the basilar artery upon topical application. Arterial constriction by this polypeptide was of greater magnitude than constriction produced by either serotonin or angiotensin.

Renewed interest in serotonin as the etiologic agent responsible for the propagation and maintenance of cerebral vasospasm after subarachnoid hemorrhage has been stimulated by the investigations of Zervas (115) and Allen, et al. (116). Zervas demonstrated prolonged intense angiographical vasospasm of the basilar artery by injection of autogenous blood into the cisterna magna in dogs. When the animals were first reserpinized and autogenous blood injected, minimal vasospasm occurred. Furthermore, in reserpinized animals which received normal homologous blood, severe vasoconstriction was demonstrated. These studies indicated that blood placed in the subarachnoid space of dogs did not cause significant spasm when the blood donor received reserpine previously, whereas blood from untreated animals caused significant vasoconstriction whether or not the recipient animal had received reserpine. Results of the study suggested that the factors primarily disposing to vasospasm were contained in the blood of the [illegible] and could be blocked by treating the donor with reserpine. The effect of reserpine in preventing arterial spasm was on blood factors rather than on the nervous elements. In a more recent study, Zervas, et al. (117) presented evidence that prevention of cerebral vasospasm induced by subarachnoid hemorrhage occurred only when blood serotonin was markedly reduced by either oral kanamycin or reserpine.

Allen et al. (116), in a comprehensive in vitro study, determined the contractile activity of various vasoactive agents on segments of canine cerebral arteries placed within a small volume chamber. Agents studied included serotonin; prostaglandins $A_1$, $E_1$, and $F_{2\alpha}$; noradrenaline; adrenaline; histamine; bradykinin; and postassium chloride. Cumulative dose-response curves were obtained for the agents tested and it was concluded from these curves and known concentrations in blood that serotonin and prostaglandin $E_1$ were the chemical regulators which, along with the sympathetic nerves, control flow under normal physiologic conditions. However, only serotonin produced a maximal contraction at a concentration known to be present free in clotted blood. From this initial study, it was concluded that serotonin was the agent in blood responsible for the cerebral arterial spasm that follows a subarachnoid hemorrhage.

In a subsequent study using the same in vitro method, the contractile activity of human serum and post-SAH cerebrospinal fluid on the canine basilar artery was tested. With chromatographic studies it was demonstrated that the majority of contractile activity in the CSF samples (collected 2 to 7 days post-SAH) was due to serotonin. Methysergide reversibly blocked the contractile response of serotonin and serum but not the activity of prostaglandin $F_{2\alpha}$. In addition, phenoxybenzamine irreversibly blocked the contractile response of serotonin, serum and post-SAH CSF, demonstrating that serotonin played a major role in the production of cerebral arterial spasm after SAH.

In their concluding in vivo canine studies it was demonstrated that physiologic concentrations of serotonin when injected intracisternally, caused cerebral arterial spasm that lasted for at least 3 hours. Comparable spasm was produced by injection of blood containing approximately the same amount of serotonin. Phenoxybenzamine reversed both the spasm produced by pure serotonin and that produced by blood.

From these detailed studies the authors proposed that cerebral vasospasm was related directly to the amount of free serotonin present in the CSF and within the clot surrounding the cerebral arteries after SAH. Prolonged vasospasm was caused by gradual release of serotonin bound in platelets (both within clots and suspended in CSF) over a period of several days as the clots lyse and the suspended platelets break down.

(b) Prostaglandin Studies

The prostaglandins (PG's), a family of naturally occurring structurally related hydroxy and hydroxy-keto unsaturated carbonylic acids, were first independently studied by Goldblatt (118) and von Euler (119) in 1933 and 1934. Bergstrom and his co-workers (120) isolated and chemically characterized the prostaglandins. Today the prostaglandins comprise at least 13 related compounds and are subdivided into groups designated by the letters E,F,A,B,C,D.

Numerous recent studies have revealed that prostaglandins are present ubiquitously and exert potent pharmacological actions on different organs and tissues. Although the precise roles of prostaglandins in health and disease remain uncertain, it appears that they may modulate local biochemical functions as well as regional circulations and may influence the physiological functions of hormones and neurotransmitters.

Prostaglandin $E_1$ has been shown to directly depress the smooth muscle of resistance type arterioles, causing vasodilation (121,122). Denton, et al.(123) assessed the cerebral vascular responses in dogs and monkeys to $PGE_1$, $PGA_1$ and $PGF_{2\alpha}$ by means of a standard perfusion technique. Prostaglandin $E_1$ depressed cerebrovascular tone in the dog but not in the monkey, whereas $PGF_{2\alpha}$ caused a marked vasoconstriction of cerebral vessels in both species. These workers postulated that prostaglandins may influence physiological and pathological cerebrovascular phenomena and under some circumstances prostaglandin $F_{2\alpha}$ may play a significant role in protracted vasospasm and prostaglandin $E_1$ in its eventual lysis. In a subsequent study from the same laboratory (124) an angiographical analysis of experimental cerebral vasospasm was performed in dogs. A statistically significant difference was found between the incidence of vasospasm obtained with injection of blood alone (33 percent) and vasospasm induced with blood and prostaglandin $F_{2\alpha}$ (92 percent). In addition, cerebral vasospasm was demonstrated with intracisternal injection of prostaglandin $F_{2\alpha}$ alone, whereas prostaglandin $E_1$ had no effect on arterial reactivity. These findings correlated with Denton's hypothesis and suggested that prostaglandin $F_{2\alpha}$ may play a role in the genesis of cerebral vasospasm seen clinically after subarachnoid hemorrhage.

Steiner, et al. (125) studied the effect of intracarotid injection of prostaglandin $E_1$ on cerebral hemodynamics in patients suffering from subarachnoid hemorrhage and all presenting with angiographical evidence of vasospasm. Arterial diameters (angiography), cerebral blood flow ($^{133}$Xenon technique)and circulation times were measured after administration of prostaglandin $E_1$. Arterial spasm and cerebral hemodynamics were not influenced by prostaglandin $E_1$. Pelofsky, et al. (126) studied the effects of intracarotid injection of prostaglandin $E_1$ upon carotid blood flow and cerebral arterial caliber (angiography) in three baboons subjected to subarachnoid hemorrhage. In this small series, prostaglandin $E_1$ relieved cerebral vasospasm and increased carotid artery blood flow.

Yamamoto, Feindel and their associates (127) studied the effects of prostaglandins $E_1$ and $F_{2\alpha}$ on the cerebral circulation in dogs by fluorescein angiography, measurement of micro-regional cerebral blood flow ($^{133}$Xenon clearance technique), and by measurement of exposed epicerebral vessels. Intracarotid injection of prostaglandin $E_1$ (0.5 mg/min) caused a vasoconstriction of the small epicerebral arteries (less then 700u diameter) and significantly reduced regional cerebral blood flow. The intense vasoconstriction was abolished by addition of ethanol to the prostaglandin $E_1$ solution. Intracarotid infusion of prostaglandin $F_{2\alpha}$ (25 mg/min) produced a selective vasoconstriction in epicerebral arteries(less than 200u diameter) and a moderate reduction in regional cerebral blood flow. These findings indicated that prostaglandins $E_1$ and $F_{2\alpha}$ , rather than being possible therapeutic agents for alleviation of cerebral vasospasm as suggested by earlier studies, were most probably involved in the pathogenesis of vasospasm.

At the present time, the controversy regarding the fundamental actions of prostaglandins has not been resolved. Factors responsible for the discrepant conclusions include: (i) species variations, (ii) anatomical variations of the carotid and intracranial arterial system, (iii) variation in surgical preparation, (iv) routes of administration of drugs, (v) dosage variability, (vi) single injection opposed to continuous infusion of drugs and (vii) techniques utilized to monitor cerebral perfusion. As stated in the Lancet Editorial (128): "If the chemistry of the prostaglandins seems complicated, the number and diversity of their reported actions may leave the uninitiated gasping."

## C. Neurogenic Factors

The role of the autonomic perivascular nerve plexuses in the genesis of cerebral vasospasm remains to be fully elucidated. Histochemical and electron microscopic techniques have confirmed the presence of noradrenergic and cholinergic nerve fibers within the adventitia of larger cerebral arteries. Furthermore, the presence of alpha- and beta-adrenergic receptor sites in the cerebral vascular bed in various species of animals has been indirectly confirmed by adrenomimetic perfusion studies (129, 130, 131).

Fraser, et al. (129), using the catecholamine fluorescent technique, demonstrated a marked reduction or complete absence of catecholamine fluorescence in the periarterial nerves after cerebral vasospasm was induced by application of autogeneous blood to the vessels. These findings suggested the possibility of noradrenergic mediation of cerebral vasospasm. Pharmacologic depletion (tyramine) of the noradrenaline stores of this plexus produced a dilated vessel but blood-induced spasm was not prevented. Alpha-adrenergic blockade (phenoxybenzamine) at the receptor prevented the induction of vasospasm by blood and reversed induced spasm. These data suggested that cerebral vasospasm may be produced by substances acting at the alpha-adrenergic receptor of the vessel wall and that blood contains a vasoconstrictor substance capable of acting at the receptor site. Peerless and Yasargil (29) demonstrated the depletion of cetecholamines in the periarterial nerves in rabbits subjected to subarachnoid hemorrhage. It was noted that in surviving animals the fluorescence intensity in the varicose terminals did not return to normal control levels for up to 3 weeks. Wilkins (132) studied the effect of parenterally administered phenoxybenzamine on vasospasm induced in dogs by injection of whole blood into the chiasmatic cistern. Intracarotid infusion of the vasoactive agent was found to be more effective in reducing vasospasm than intravenous administration, but was accompanied by a high mortality rate.

The capability of alpha-adrenergic blocking agents to prevent or alleviate cerebrovascular spasm produced by application of blood to the basilar artery in cats was investigated by Flamm, et al. (133). Intravenous phenoxybenzamine was effective in preventing spasm, provided at least one and one half hours elapsed prior to application of blood. Topical application of the drug prevented spasm and also relieved the induced spasm.

Preliminary clinical studies have been undertaken to stuay the efficacy of alpha-adrenergic blocking agents in the treatment of patients with cerebral vasospasm. Cumming and Griffith (134) administered phenoxybenzamine by intracarotid injection in patients with severe cerebral arterial spasm and reported a rapid improvement in their neurological deficits. Impressed by their preliminary findings, these investigators have administered phenoxybenzamine routinely after aneurysmal clipping procedures. No deleterious effects from the medication have been encountered. Takamatsu, et al. (135) reported two patients in whom severe vasospasm was reversed by intracarotid injection of phenoxybenzamine.

Contrary to the above clinical investigations, Handa, et al. (136) were unable to reverse cerebral vasospasm in two patients with subarachnoid hemorrhage by intracarotid phenoxybenzamine injections. In their third patient with vasospasm, phenoxybenzamine caused a marked decreased in cerebral blood flow as determined by the $^{133}$Xenon clearance method.

Because the etiology of cerebral vasospasm has not been fully elucidated at the present time, the treatment of pathological vasoconstriction remains unsatisfactory. Clinical and experimental investigations of intracranial arterial spasm must all be interpreted with caution and the following criteria, as outlined by Wilkins (132), must be considered:

(i) Similar stimuli produce different arterial responses in different species, thus results of animal experimentation cannot be directly applied to man.

(ii) Intracranial spasm appears to be biphasic in nature. The mechanisms responsible for the acute phase may be different from those causing chronic spasm. Success in preventing or ameliorating one phase may be unsucessful in affecting the other phase.

(iii) An exact reproduction of human subarachnoid hemorrhage has not been developed.

(iv) Cerebral arterial spasm can be produced by various stimuli, each apparently having a different mechanism.

(v) Current angiographical techniques are not capable of detecting changes in the cerebral microcirculation.

(vi) Cerebral arterial spasm may represent a protective mechanism and attempts at treatment of spasm may be harmful under certain circumstances. Furthermore, pharmacologic vasodilatory agents may cause systemic hypotension and reduction in cerebral perfusion, especially when cerebral autoregulation is impaired. As well, arteries and arterioles in an ischemic area may respond differently to drugs and gases than those in a normal area.

Even with the limitations and precautions outlined above, certain agents do appear promising in experimental and clinical studies. However, a more systematized experience is required with chronic animal experiments and then with humans, employing various drug dosages, dosage schedules and routes of administration.

# CHAPTER THREE

## MATERIALS AND METHODS

### I. ANIMAL PREPARATION

Juvenile and adult female rhesus monkeys (macaca mulatta) weighing between 2.0 and 4.5 kilograms were utilized in the entire study. In the initial experiments (study A), sedation was achieved with intraperitoneal sodium pentobarbatal (30-75 mg/kg) and in the subsequent studies (studies B and C), with intravenous injection in doses ranging from 20-30 mg/kg. Flexometallic endotracheal tubes were introduced and the animals were ventilated with a Harvard variable phase mechanical respirator. Paralysis of the animals was induced with intravenous d-tubocurarine (induction dosage 0.9 mg/kg)and maintained with additional supplements (0.3 mg/kg)as required. General anesthesia was maintained throughout the experimental period with a mixture of nitrous oxide ($N_2O$) and oxygen ($O_2$) from a reservoir in a ratio of 2:1 (1.0 liter $N_2O$ to 0.5 liters $O_2$ per minute).

Body temperature was continuously monitored by an esophageal thermometer (tele-Thermometer - Yellow Springs Instrument Co.) and maintained between 36 and 38 degrees centigrade by a 250 watt infra-red light bulb positioned above the animals. Standard lead electrocardiography (Beckman Dynograph (typeR) Recorder) was performed in all animals and in several animals, electrical activity of the brain was monoitored through eight needle or disk electrodes by an encephalograph recorder (Grass model 6 Encephalograph).

## II. SURGICAL PREPARATION

Femoral artery catheterization was performed in the anesthetized animals and arterial blood samples were immediately obtained for pH and blood gas analysis (Instrument Laboratory Inc., pH/ Gas Analyzer, model 113). By adjusting the volume output from the respirator, arterial pH (apH) carbon dioxide ($PaCO_2$) and oxygen ($PaO_2$) values were kept within the physiological range during surgery. Thereafter, arterial pH and blood gas analysis was performed during each measurement of cerebral blood flow. Hemoglobin and hematocrit determinations, required for CBF calculation, were carried out at the onset, midpoint and upon termination of each experiment. Mean arterial blood pressure (MaBP) was continuously measured by a pressure transducer (Statham (P23Db) Transducer) connected to the femoral artery catheter. Maintenance fluids and drugs (d-tubocurarine, heparin, atropine and prostigmine) were administered as required through a cannula positioned in the femoral vein.

A cranial twist-drill hole (1.5 mm diameter) 0.5 to 1.0 centimeters dorsal to the nasion was performed in the animals to be subjected to intracranial hemorrhage. Hemostasis was achieved with bone wax application and the defect was sealed until the time of induction of the intracranial hemorrhage. In several animals a Numoto electrical pressure switch (Ladd Research Industries Inc.) for intracranial pressure measurement was implanted extradurally through a 1.25 centimeter burr hole positioned immediately lateral to the midline and posterior to the coronal suture. Upon implantation, the bone plug was repositioned, the defect sealed with dental cement and the scalp incision was sutured.

Cervical dissection to the common carotid artery bifurcation was performed with the aid of an operating microscope (Ziess Instrument Company) and precaution was taken to preserve the hypoglossal nerve and the peri-arterial autonomic nerve plexus. The external carotid artery was isolated and doubly ligated immediately distal to the origin of the external maxillary (facial) artery. The external maxillary artery (largest branch of the external carotid system in the monkey) was carefully dissected from surrounding tissues and doubly ligated approximately two centimeters distal to its origin. A 21-gauge polyethylene catheter was inserted in a retrograde manner into the origin of the internal carotid artery. On occasion, internal carotid catheterization was facilitated by distal division and mobilization of the external maxillary artery. After adequate placement within the internal carotid artery (with the aid of fluoroscopy), the catheter was secured with a ligature around the proximal segment of the external maxillary artery. The catheter was connected to a three-way stopcock through which heparinized normal saline was slowly infused.

## III. METHOD OF SIMULATING INTRACRANIAL HEMORRHAGE

Prior to the induction of subarachnoid hemorrhage, a circumferentially beveled, gauge 19 spinal needle was carefully inserted (under fluoroscopy) along the floor of the anterior fossa into the subarachnoid space dorsal to the planum sphenoidale or into the chiasmatic cistern. Adequate placement of the needle was confirmed with return of clear cerebrospinal fluid (CSF) after removal of the needle stylette. With the needle secured to the skull by a screw device a CBF and angiographical study was routinely carried out to determine the effect of needle insertion on cerebral perfusion and intradural arterial diameters. Subarachnoid hemorrhage was induced by manual injection of four milliliters of fresh autogenous arterial blood under constant pressure over a twenty-second interval. Subdural hemorrhage was induced by the same procedure, except that blood was injected into the pre-chiasmatic subdural space. Following intracranial hemorrhage induction, post-hemorrhage CBF studies were begun within three minutes and followed by angiographical studies (approximately twenty minutes post-hemorrhage). Thereafter, CBF studies were performed at approximately thirty-minute intervals for a three-hour period while additional angiographical studies were performed at ninety minutes and upon termination of the experiments (3 hours).

## IV. HANDLING AND DISPENSING $^{133}$XENON

$^{133}$Xenon gas, an inexpensive and readily available radiochemical with a half-life of 5.3 days, was obtained at weekly intervals in break-seal vials (1.0 Curie) from the Oak Ridge National Laboratory (Oak Ridge, Tennessee, U.S.A.). Since the aqueous form of $^{133}$Xenon was required for intra-arterial CBF determination, a special handling and dispensing system with a high transfer efficiency (nearly 99 percent) was constructed in our laboratory (137). During the transference of 1.0 Ci gaseous $^{133}$Xenon into aqueous solution, a procedure requiring five to eight minutes, the maximum radiation dose to the operator at a distance of one meter was approximately 1 mR/hour. Dispensing of individual doses of aqueous $^{133}$Xenon (0.5 to 3 mCi) required approximately one minute and involved no direct handling of unshielded activity. Glass syringes were routinely utilized for dispensing the

$^{133}$Xenon solution and these were immediately placed inside lead tubes.

## V. ADMINISTRATION AND DETECTION OF THE RADIOISOTOPE

Cerebral blood flow was measured by the $^{133}$Xenon intra-arterial injection (residue detection) method developed by Lassen, Ingvar and their associates.

In study A (determination of partial hemispheric blood flow (pHBF)), 0.5 to 1.0 millicurie of $^{133}$Xenon, dissolved in 0.5 milliliters sterile saline, was injected over a 2 to 3 second interval into the internal carotid artery. The clearance of $^{133}$Xenon from the brain was simultaneously measured for eleven minutes by two 2.54 centimeter diameter, 1.27 centimeter thick, integral line sodium-iodide-thallium activated crystals placed symmetrically over the right and left parietal regions (Harshaw Chemical Co.). These scintillation detectors were collimated with cylindrical lead tubes, 11 centimeters long and 1.50 centimeters thick. The inner diameter of each collimator was 2.54 centimeters. The isoresponse curve for the collimated detector, determined with a point source of $^{133}$Xenon in water is shown in Figure 1.

The signal output from each detector (sampling time of 4 seconds) was amplified, analyzed and stored via multiplexed high speed time-sequenced inputs (buffered device) into a 400-word magnetic core memory. The electronic circuitry of the system is illustrated in Figure 2. Punched paper tape data output from the 400-word memory allowed immediate access to calculated flows via an on-site laboratory digital computer (Hewlett-Packard 2116B computer).

Background radiation (remaining activity) was recorded for a

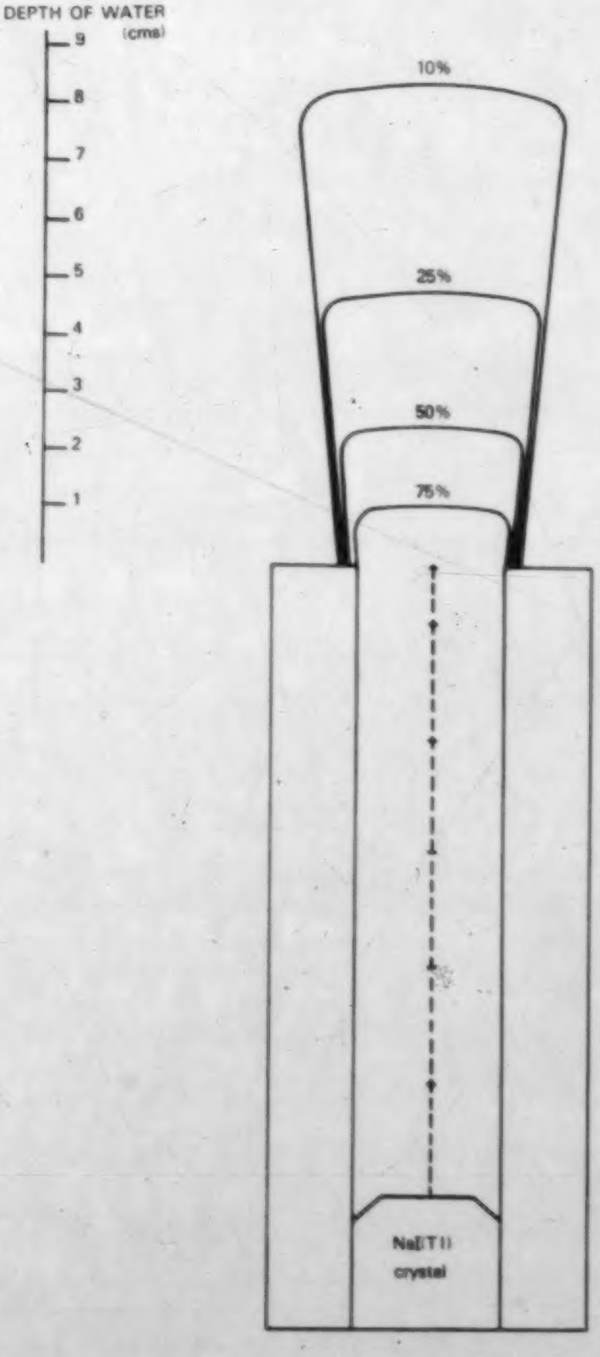


Figure 1: Collimated isoresponse curves with a point source of 133xenon in water. Isocount contours at the 75, 50, 25 and 10 percent response levels.

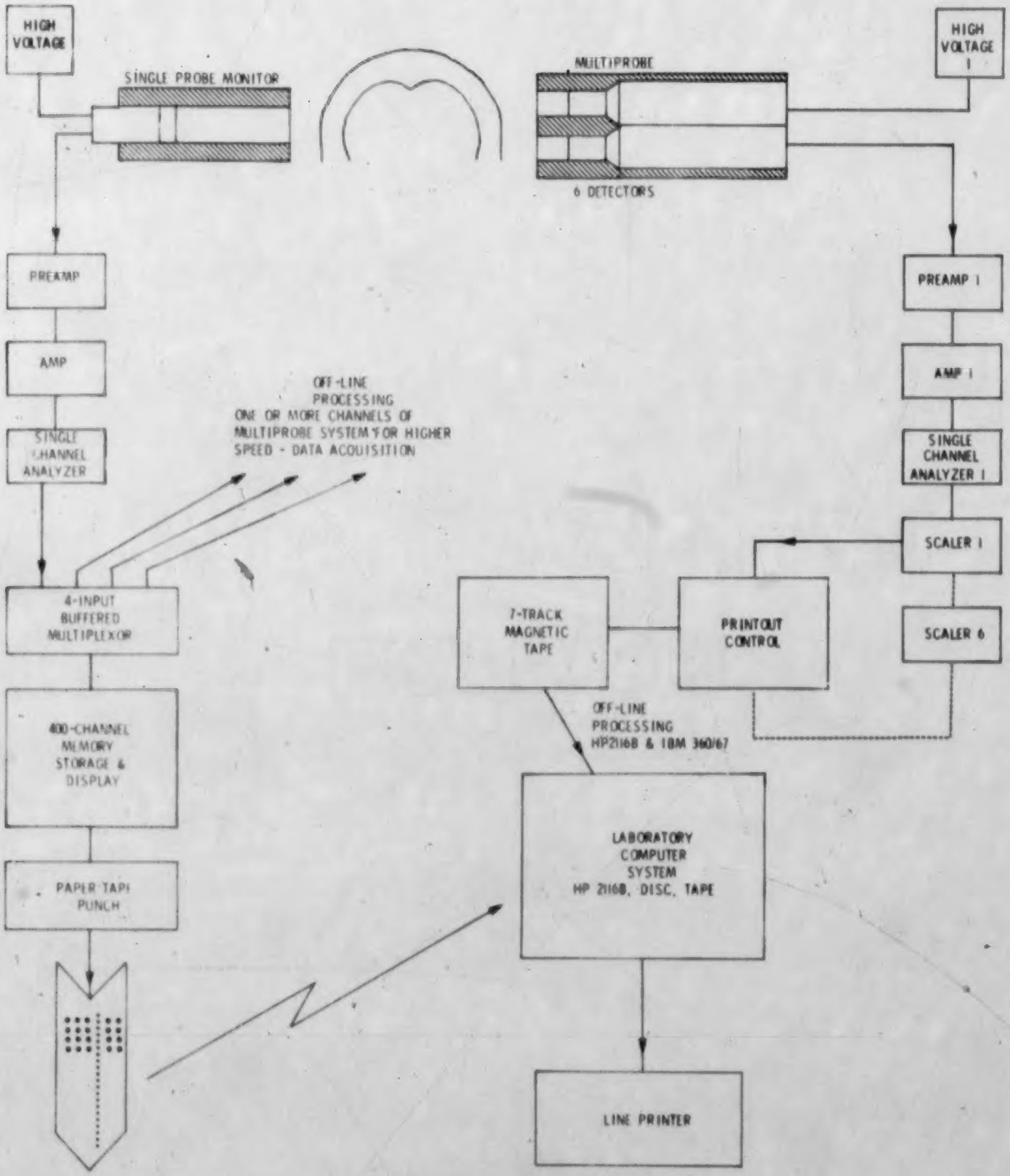

HIGH VOLTAGE
SINGLE PROBE MONITOR
MULTIPROBE
HIGH VOLTAGE I
6 DETECTORS
PREAMP
AMP
SINGLE CHANNEL ANALYZER
OFF-LINE PROCESSING
ONE OR MORE CHANNELS OF MULTIPROBE SYSTEM FOR HIGHER SPEED - DATA ACQUISITION
PREAMP I
AMP I
SINGLE CHANNEL ANALYZER I
SCALER I
SCALER 6
4-INPUT BUFFERED MULTIPLEXOR
7-TRACK MAGNETIC TAPE
PRINTOUT CONTROL
400-CHANNEL MEMORY STORAGE & DISPLAY
OFF-LINE PROCESSING HP2116B & IBM 360/67
LABORATORY COMPUTER SYSTEM HP 2116B, DISC, TAPE
PAPER TAPE PUNCH
LINE PRINTER

**FIGURE 2:**

**INSTRUMENTATION FOR PARTIAL HEMISPHERIC AND REGIONAL CEREBRAL BLOOD FLOW STUDIES**

two-minute period and a calculated mean value was subtracted from the washout curves prior to computer calculation of partial hemispheric blood flows.

In studies B and C, regional clearance rates for $^{133}Xe$ were measured by a six-detector scintillation counter assembly constructed in this laboratory. The detector system (figure 3) was designed to record the radioactivity in each of five discrete cylindrical volumes of unilateral brain tissue, with the sixth detector recording radioactivity in the unilateral orbito-maxillary tissues (Figure 4). Each scintillation counter comprised of 0.60 centimeter diameter, 1.25 centimeter thick sodium-iodide-thallium activated crystal (NaI(Tl)) coupled to a 1.25 centimeter diameter photomultiplier (Phillips XP 101) with a truncated plexiglass light guide (Figure 5). The detectors, spaced at a distance of 1 centimeter from each other, were mounted in a stainless steel collimator block with a front face of each crystal recessed 7.5 centimeters from the block face. An additional 1.5 centimeter lead collimator applied to the block face ensured measurement of radioactivity from discrete volumes of brain tissue. The isoresponse curves for two adjacent collimated detectors are shown in Figure 6.

Pulses from each detector were amplified and then input to single-channel analyzers which accepted pulses equivalent to the energy range 70 to 90 Kev. The output from each single-channel analyzer was recorded in a high speed buffered scaler which read out onto a seven track digital magnetic tape. A sampling time of four seconds was utilized and the data was processed by an on-site HP2116B digital computer. Partial hemispheric blood flow from the contralateral parietal area was simultaneously measured by the method described in the

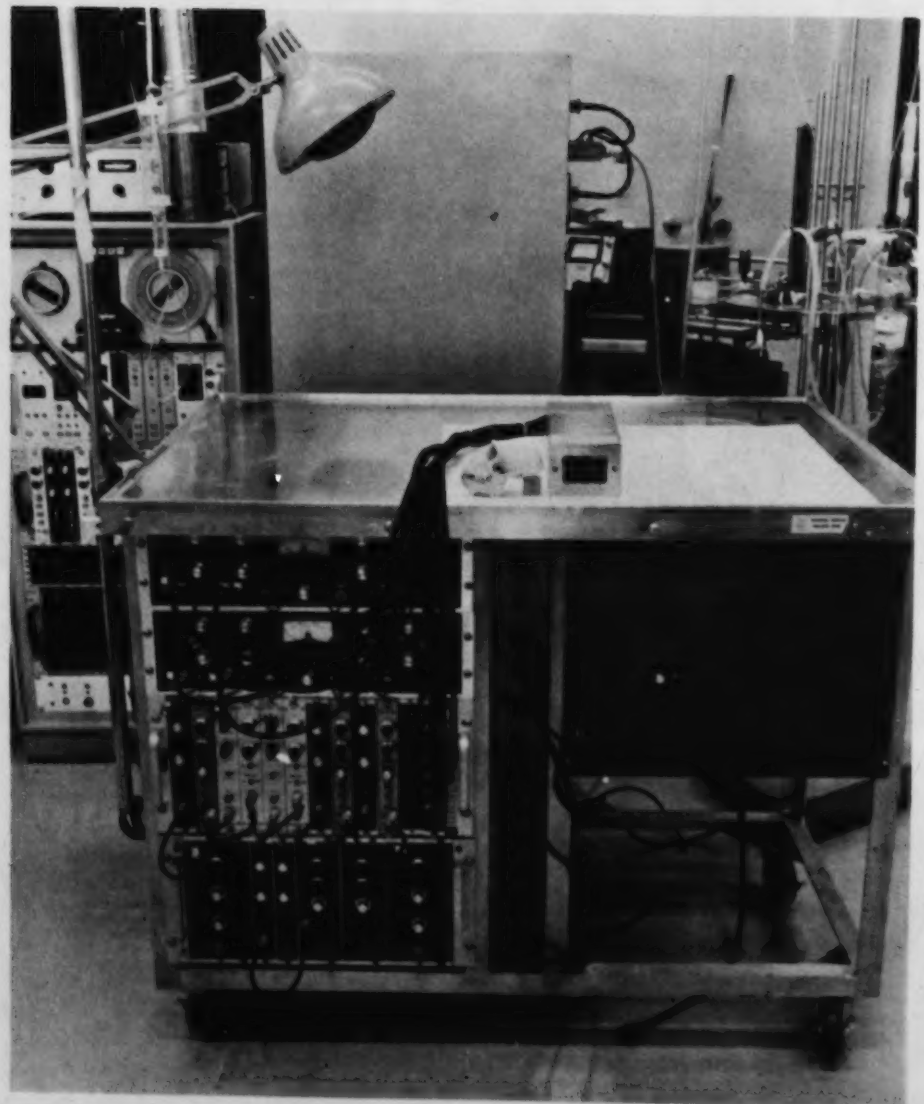


Figure 3: Six detector scintillation counter system.

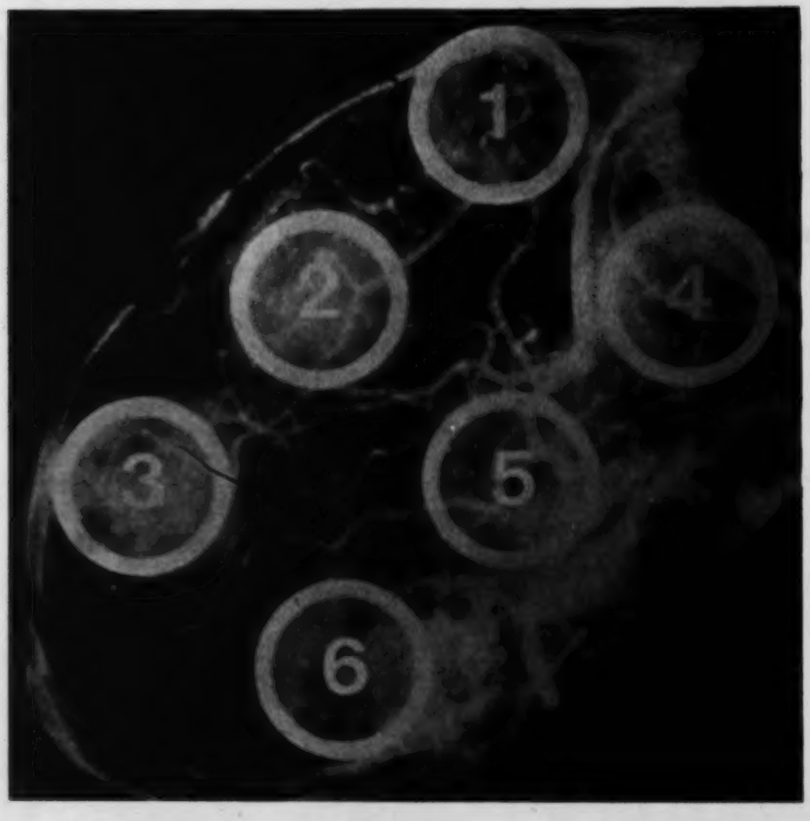


Figure 4:Lateral cerebral angiogram showing scintillation detector placement. Detectors 1, 2, 3 and 5 measure rCBF from the frontal, central, parietal and temporal areas of the brain. Cerebellar and orbito-maxillary perfusion is measured by detectors 6 and 4 respectively.

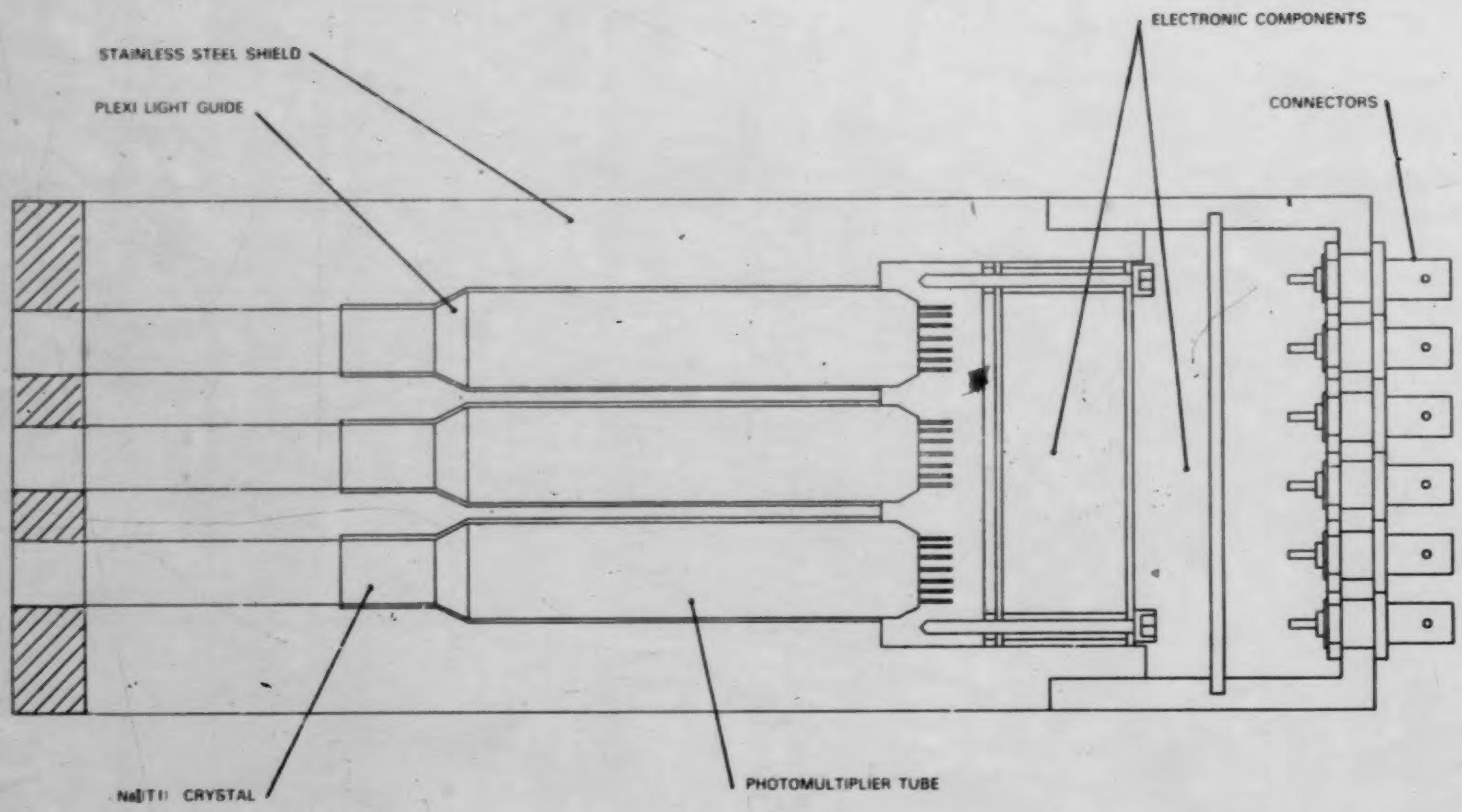


Figure 5: Multidetector System (Superior view).

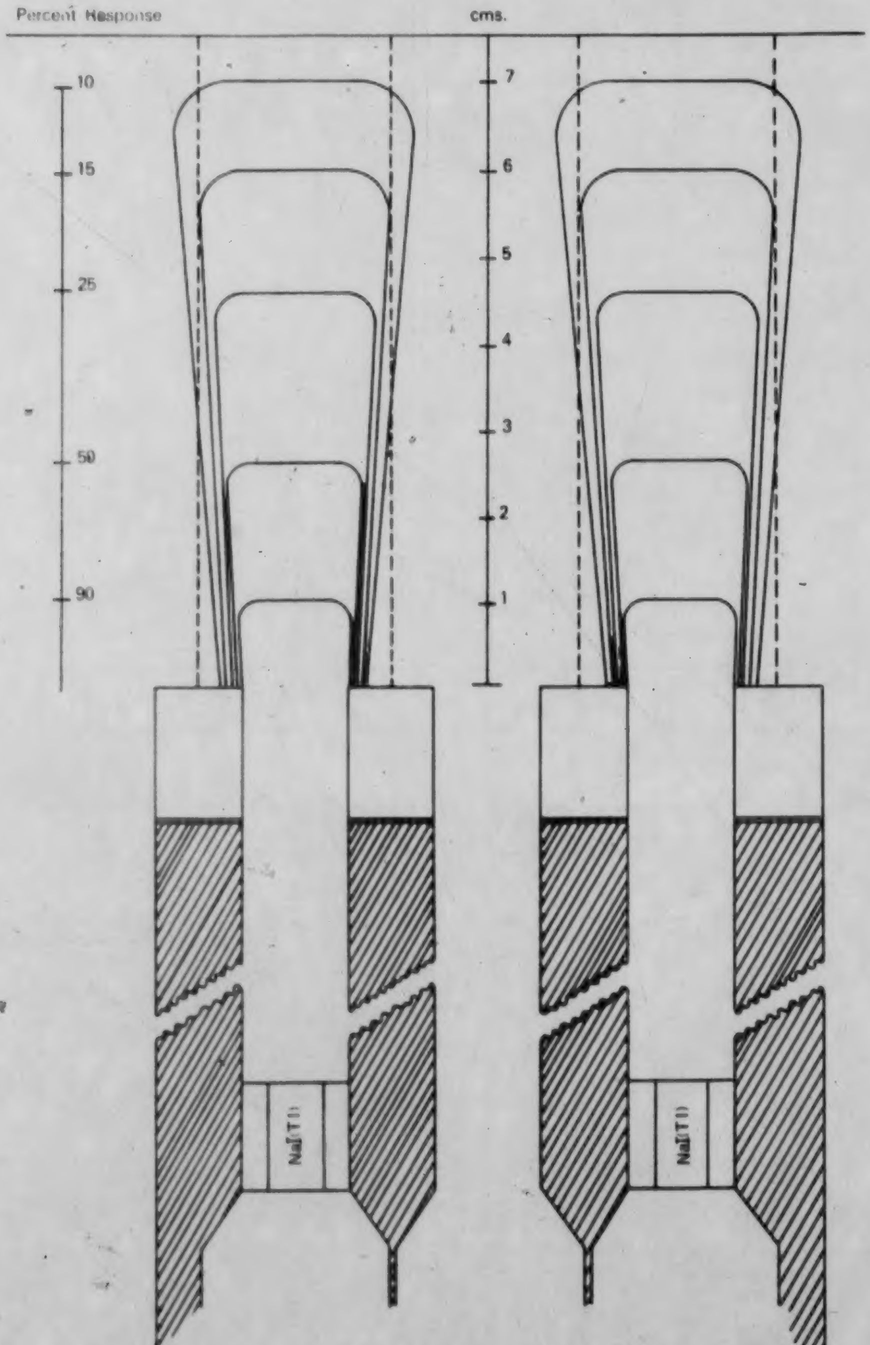


Figure 6: Isoresponse curves for two adjacent collimated detectors with a point source of 133xenon in water. Isocount contour at the 90, 50, 25, 15 and 10 percent response levels.

initial study.

Correct placement of the multidetector system was achieved by applying a plastic template (with six radiopaque circular markers with identical diameters as the detectors) to the lateral surface of the skull. Manipulation of the monkey's head under fluoroscopy ensured adequate positioning of the multidetector system (Figure 4).

Regional cerebral blood flow was measured from the washout curves after internal carotid injection of 3.0 to 3.5 mCi $^{133}$Xe dissolved in 0.25 to 0.5 milliliters sterile saline. Ipsilateral extracerebral contamination with $^{133}$Xe was minimized by ligation of the external carotid artery. Mean hemispheric blood flow (mHBF) was determined by averaging flows from the four supratentorially placed detectors (probes 1, 2, 3, and 5).

## VI. CALCULATION OF CEREBRAL BLOOD FLOW

Cerebral blood flow was calculated by the biexponential (compartmental) stochastic (height over area) and the initial-slope-index methods for the biological clearance curve. A detailed discussion of the three methods of CBF calculation has been presented and will only be reviewed here.

In the compartmental analysis, it is assumed that the semi-logarithmic replot of the clearance curve of $^{133}$Xe can be represented by two exponential components. From these two phases, blood flow in the grey ($f_g$) and white ($f_w$) matter are determined from the intercepts of the two slopes at zero time. Knowing the perfusion rates and the relative weights of each tissue, the weighted mean blood flow for the cerebral tissue under observation can be calculated from the equation:

$$\bar{f}_C = W_g \cdot f_g + W_w \cdot f_w \text{ ml/100gm/min} \qquad \text{-B14}$$

The stochastic method formulated mathematically by Zierler can be applied to calculate the mean flow values from the linear washout curve according to equation B22.

$$\text{rCBF} = \frac{(H_0 - H_{10})}{A_{10} - \text{Background}} \cdot \lambda \cdot 100 \text{ ml/100gm/min} \qquad \text{-B22}$$

where $H_0$ corresponds to the total number of indicator particles to be cleared and $H_{10}$ the level of the clearance curve at 10 minutes. $A_{10}$ is determined by integrating the counts recorded during 10 minutes and $\lambda$ denotes the mean tissue: blood solubility coefficient for $^{133}$Xenon.

The initial-slope-index (ISI) method utilizes the calculated slope of the first 1.5 to 2.0 minutes of the logarithmic clearance curve. An estimate of the mean CBF can be readily determined using:

$$\text{rCBF}_{ISI} = 100\lambda_g \cdot 2.30\ D_{\text{initial}} \text{ ml/100gm/min} \qquad \text{-B26}$$

where $\lambda_g$ corresponds to the $^{133}$Xenon partition coefficient of grey matter and D represents the numerical value of the slope of the curve.

Because of the profound influence of carbon dioxide on cerebral perfusion, particular care was taken to maintain $PaCO_2$ levels within the physiological range (40-45mm Hg) during steady state studies. Both uncorrected and corrected flows for $PaCO_2$ were calculated. For each millimeter change in $PaCO_2$, a correction factor of 2.5 percent for CBF change in the same direction was incorporated into the computer program (138). In the post-hemorrhage period, special care was taken to keep $PaCO_2$ values near 40 mm Hg since the effect of cerebral apoplexy on carbon dioxide responsiveness has not been adequately determined.

The partition coefficient for $^{133}$Xe in the grey and white matter was calculated according to the method of Veall and Mallett (139) using the solubilities of $^{133}$Xe in the grey and white matter of baboons (25). Since the hematocrit influences the solubility coefficient of $^{133}$Xe in whole blood and the blood-brain partition coefficient, a correction equation as described by Veall and Mallett (139) was incorporated into CBF calculation. Hematocrit determinations were routinely performed at the onset, midpoint and upon termination of each experiment. A mean partition coefficient for the brain was calculated on the basis of a 52:48 grey to white matter ratio as determined for the baboon brain by James (25).

The calculation of CBF presupposes that the recorded clearance curve represents a single transit of the tracer and that the tracer does not recirculate to the counting field. Even though the lungs function as an effective filter for inert gases with low solubility (90 percent of $^{133}$Xe is cleared during the first passage through the lungs and the remaining 10 percent is distributed in the total cardiac output with only 14 percent of the recirculating activity distributed to the brain) recirculation does occur. However, only in the presence of gross pulmonary insufficiency or with a rebreathing ventilation system does recirculation become a significant factor in CBF determination (138). In the present experiments, exhaled $^{133}$Xe was removed by a hose at a slight negative pressure to the respirator. Correction for recirculation was not performed.

## VII. ANGIOGRAPHICAL STUDIES

Cerebral angiographical studies were performed in control and experimental animals in studies B and C by forceful injection of 1.0 to 1.5 milliliters Meglumine iothalamate (Conray 60) into the internal carotid artery. Only lateral angiograms were obtained and care was taken to maintain magnification factors constant during each experiment. Angiography was routinely carried out after completion of a cerebral blood flow study.

Angiograms showing the arterial phase most distinctly were selected for measurement studies. Only the large intradural capacitance arteries, i.e., intradural internal carotid (IDICA), middle cerebral (MCA), proximal pericallosal (PCA), and distal pericallosal (DPA) were measured (Figure 7). Arterial vessel (intra-luminal) diameters at predetermined fixed locations were measured with a micrometer (Vernier calibrated) lens system positioned at a fixed focal distance from the film (Figure 8). Usually three to five films from each angiographical sitting were selected and the vessels of each film were measured three times. The mean diameter value (and standard deviation) for each artery was therefore obtained from nine separate readings and this value was utilized for statistical analysis.

## VIII. MEASUREMENT OF INTRACRANIAL PRESSURE

Intracranial pressure (ICP) was continuously measured in several animals by a Numoto electrical pressure switch implanted through a burr hole into the extradural space. The calibration and intracranial pressure measurement systems are demonstrated in Figure 9.

Prior to implantation, the recording system was calibrated (in millimeters mercury) with a manometer. With the pressure switch in air, calibration to a atmospheric pressure (baseline zero value) was rapidly achieved by manually altering the quantity of water (via syringe A) within the external manometric reservoir and maintained

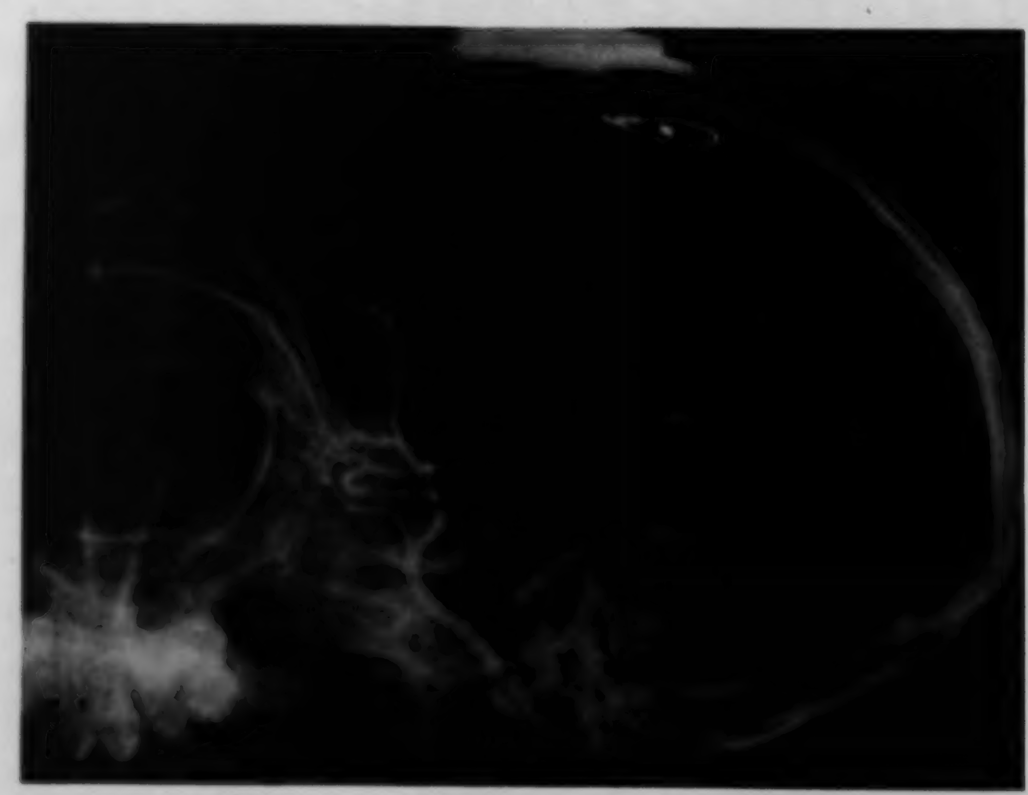


Figure 7: Lateral angiogram showing predetermined fixed locations of arterial diameter measurement.

Figure 8: Micrometer lens system (vernier calibrated).

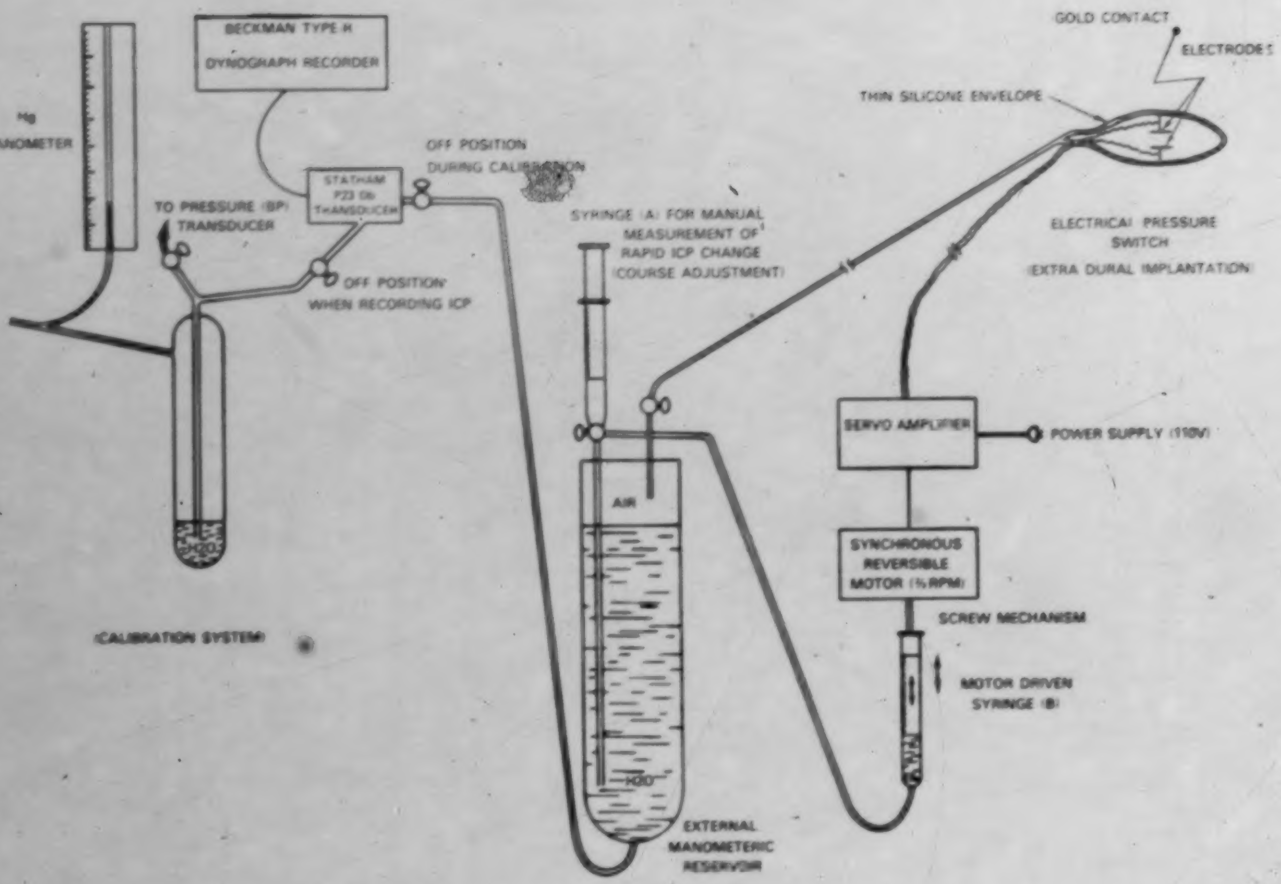


Figure 9: Intracranial pressure calibration and measurement system.

(via syringe B) by reversible servo-unit-motor mechanism. After airtight implantation of the pressure switch, intracranial pressure was continuously measured by the reversible servo-unit-motor mechanism. During induction of intracranial hemorrhage, the apparatus was manually operated (via Syringe A) and large rapid changes in intracranial pressure were measured. Thereafter, the system operated automatically.

At the termination of each experiment the zero (baseline) drift was determined with the pressure switch in air and incorporated into the calculation of the true intracranial pressure.

## IX. NEUROLOGICAL ASSESSMENT

Neurological examination was performed in all monkeys in the two to three hour period after anesthesia was discontinued. A five division neurological grading system was utilized for evaluation of the animals.

Grade I: alert, active and vocal, no evidence of neurological deficit, accept food and water.

Grade II: mildly obtunded, not as active or vocal, no significant neurological deficit.

Grade III: moderately obtunded, neurological deficit (i.e., hemiparesis, paraparesis, cranial nerve palsy), usually assume a semi-supine position but sit up when stimulated, respond to all forms of stimulation (auditory, touch, pain).

Grade IV: Severely obtunded, severe neurological deficit (i.e., hemiplegia, quadraplegia), little or no response to painful stimulation, frequently exhibit generalized intermittent clonic seizures of variable duration.

Grade V: moribund, unresponsive to all forms of stimulation, failing vital signs (falling BP, arrhythmias, shallow irregular respirations).

## X. EXPERIMENTAL DESIGN

### A. Study A: The Effect Of Induced SAH And Subarachnoid Saline Injection On CBF, Neurological Status And Other Physiological Parameters

A total of twenty-two monkeys were utilized. Four monkeys served as controls and had repeated CBF studies (partial hemispheric blood flow) performed at 30 minute intervals for five-hour period. Four animals received 4 milliliters of body temperature normal saline (pH 6.1) injected into the subarachnoid space and fourteen monkeys were subjected to subarachnoid hemorrhage. Three to six CBF determinations at 30-minute intervals were made prior to injection of saline or whole autogenous blood into the subarachnoid space. Post-injection CBF measurements were started within 3 minutes following the injection and continued at 30-minute intervals for a period of three hours. Every effort was made to maintain the $PaCO_2$ within the physiologic range. Following the final CBF study, anesthesia was discontinued and d-tubocurarine was reversed with atropine (0.05 mg) and neostigmine (0.2mg). The animals were observed over the next two to three hours as they recovered from anesthesia and their neurological status was evaluated at the end of this time. The animals subjected to the subarachnoid hemorrhage were sacrificed with an overdose of sodium pentobarbatal and post mortem examination was carried out. Animals that showed significant subdural or intracerebral hemorrhage were excluded from the series. The brains were photographed and thick brain slices were examined grossly.

### B. Study B: The effect Of SAH And SDH On Regional Cerebral Blood Flow, Intradural Arterial Caliber And Neurological Status.

Of the 21 monkeys utilized in this study, six served as controls and had repeated rCBF and pHBF studies performed at 30-minute intervals for a 5- to 6-hour period. Fifteen animals were subjected to intracranial hemorrhage. Subarachnoid hemorrhage was induced in eight, pure subdural hemorrhage in two, and combined SDH:SAH in five other monkeys. Usually three to four baseline rCBF determinations at 30-minute intervals were made prior to needle insertion into the subarachnoid or subdural space. After correct needle placement, angiography was carried out and was followed by a final baseline rCBF study. Insertion of the needle into the subdural or subarachnoid space did not cause alteration in CBF or vessel caliber. Post-hemorrhage rCBF studies were started within 3 minutes of the hemorrhage and continued at 30-minute intervals for a period of 3 hours. In one study, rCBF and angiography were performed at 24 hours post-hemorrhage.

In the control series, angiograms were performed on completion of surgery and prior to termination of the experiments. In monkeys subjected to hemorrhage, baseline angiograms were carried out at the onset of the experiments and upon insertion of the needle into the chiasmatic area. Post-hemorrhage angiograms were performed at 20,90 and 180 minutes.

Following the three hour post-hemorrhage period, anesthesia was discontinued and the animals were reversed with atropine and neostigmine. The animals were observed over the next two hours and their clinical and neurological state was evaluated. The type, degree and location of hemorrhage was determined by gross pathological examination.

### C. Study C: The Effect Of Graded Hypocapnia And Hypercapnia On rCBF, Intradural Vessel Reactivity, And Neurological Status In Control Monkeys And Monkeys Subjected To SAH And TSICA

The effect of hypocapnia and hypercapnia on rCBF and intradural vessel reactivity (as determined by serial angiography) was determined in nine control monkeys over a 5- to 6-hour experimental period. At the onset of each experiment, angiography and rCBF studies were carried out during normocapnia ($PaCO_2$ 40±5 mm Hg). Usually two to four baseline rCBF studies were performed at 30-minute intervals prior to induction of hypocapnia and hypercapnia. Graded hypocapnia to $PaCO_2$ values near 20 mm Hg was achieved by increasing the volume of gas mixture delivered by the respirator. Hypercapnia was induced in a graded manner by addition of carbon dioxide gas to the anesthetic mixture until a maximum $PaCO_2$ value of 80 mm Hg was reached. Changes in $PaCO_2$ were in steps of 5 to 10 mm Hg, and 10 to 15 minutes was allowed following each change for stabilization of hemodynamic reponses. In several animals, graded hypocapnia to 20 mm Hg was induced following the baseline normocapnic studies, while in others stepwise hypercapnia was first induced. In general, angiography was performed during normocapnia at the onset of each experiment and at the extreme hypocapnic and hypercapnic states. Upon completion of each experiment the animals were neurologically assessed.

Hemodynamic responses to graded $PaCO_2$ change were examined in eight animals subjected to two forms of cerebrovascular insult: subarachnoid hemorrhage and traumatic spasm of the ipsilateral carotid artery (TSICA). In four animals cerebral hemodynamic responses (rCBF and angiographical studies) to graded $PaCO_2$ change were tested prior to and after subarachnoid hemorrhage. Hemorrhage was always induced during normocapnia. The first post-hemorrhage rCBF study was performed three minutes after the insult and was immediately followed by angiography. Usually one or two additional rCBF studies were carried out in the normocapnic state. During graded $PaCO_2$ change, rCBF was measured at approximately 30 minute intervals. Additional angiographical studies were performed whenever marked rCBF changes occurred with $PaCO_2$ change and at the extreme hypocapnic and hypercapnic values. The concurrent pre-subarachnoid and post-subarachnoid hemorrhage rCBF and vessel diameter (angiography) studies permitted correlative analysis to be performed.

Four animals displayed marked spasm of the origin of the internal carotid artery. The vasoconstriction was produced by trauma induced during catheterization and was demonstrated by angiography carried out during normocapnia at the onset of each experiment. Cerebral vessel reactivity and rCBF studies were performed after stepwise changes in $PaCO_2$ were induced. The hemodynamic responses in these animals were compared with the responses observed in the control animals.

Upon termination of each experiment, the animals were observed for two hours and neurologically assessed. They were then sacrificed and the brains removed for gross pathological examination. Histological examination was performed on two specimens (one SAH and on with TSICA).

## XI. OBJECTIVES OF EACH STUDY.

The primary objectives in each study are listed below.

### A. Study A

(a) The development of an accurate reproducible method of quantitatively measuring cerebral blood flow in the rhesus monkey.

(b) Determination of the normal range of CBF values and other physiological parameters measured during steady state conditions in the rhesus monkey.

(c) To attain a simple laboratory reproduction of subarachnoid hemorrhage.

(d) To determine the effect of SAH on CBF and other physiological parameters measured.

(e) To perform correlative studies between the changes in cerebral perfusion after SAH and the neurological state of the animals.

B. Study B

(a) To determine the interregional variation of the rCBF in control animals by a specially constructed multidetector system.

(b) To determine whether induced SAH causes regional or global changes in CBF.

(c) To examine the effect of induced SAH on intradural vessel diameters and the duration of the changes observed.

(d) To observe the effect of subdural hemorrhage on rCBF, vessel caliber and other physiological parameters.

(e) To perform correlative studies between rCBF, vessel diameter change and the neurological status of animals subjected to intracranial hemorrhage.

C. Study C

(a) To determine the effect of induced hypocapnia and hypercapnia on rCBF, intradural vessel reactivity and other physiological parameters monitored in control animals.

(b) To perform correlative studies between rCBF, vessel response and neurological state in a control series of monkeys.

(c) To determine the effect of SAH on rCBF and vessel reactivity during normocapnia, hypocapnia and hypercapnia.

(d) To determine the effect of traumatic spasm of the internal carotid artery on ipsilateral rCBF.

(e) To observe the effect of TSICA on rCBF and vessel reactivity in response to stepwise changes in $PaCO_2$.

(f) To perform correlative studies between rCBF, vessel diameters and neurological state during graded changes in $PaCO_2$ in animals subjected to SAH and TSICA.

# CHAPTER FOUR

## RESULTS

### I. STUDY A: THE EFFECT OF INDUCED SAH AND SUBARACHNOID SALINE INJECTION ON CBF, NEUROLOGICAL STATUS AND OTHER PHYSIOLOGICAL PARAMETERS

#### A. Cardiovascular and Intracranial Pressure Responses

The mean arterial blood pressure (MBP) and standard deviation of the control, pre-saline injection and pre-SAH monkeys was 100 ± 17, 116 ± 12, and 108 ± 24 mm Hg respectively. Injection of saline or blood into the SAS caused an increase in systemic blood pressure beginning at 5-10 seconds and reaching a maximal value within 30 seconds after the onset of injection. The mean peak values for the saline and SAH groups was 155 ± 11 and 177 ± 39 mm Hg. Within 5 minutes after injection, MBP values returned to pre-injection levels in both groups.

The mean heart rate (HR) of the control, pre-saline and pre-hemorrhage monkeys was 176 ± 23, 195 ± 17, and 191 ± 28 beats/minute. Decreased HR was observed within 30 seconds after injection of saline or blood into the SAS. Mean minimal values for the two groups were 120 ± 39 and 119 ± 28/min., respectively. Post-injection values beyond the first 10 minutes were not significantly different from pre-injection values.

Arrythmias were first observed 5-45 seconds from the onset of injection of blood into the SAS. Five animals showed them within 10 seconds, 5 within 11-20 seconds, 3 later than 20 seconds, and one showed no significant response. Sinus arrythmia, nodal beats, nodal rhythm and premature ventricular contractions were frequently seen. Other induced EKG changes included increased T-wave amplitude, T-wave inversion, U waves, S-T elevation and increased P-wave amplitude. Cardiac rhythm reverted to normal within 3 minutes in 9 animals, within 5 minutes in one animal, within 10 minutes in 2 animals, and with 30 minutes in one.

Three of four monkeys receiving subarachnoid saline injection demonstrated sinus arrythmias lasting up to 5 minutes. Other abnormal cardiac responses were not observe in the saline group.

The mean intracranial pressure (ICP) and standard deviation in the control,pre-saline and pre-hemorrhage monkeys were 14 ± 3, 17 ± 5, and 15 ± 6 mm Hg, respectively. The mean post-saline and post-SAH ICP peaks which occurred a few seconds after the peak BP was reached was 140 ± 11 and 151 ± 56, respectively. In most animals ICP returned to near control values within 5 minutes (Figure 10).

Hemoglobin determination at the onset of the experiments averaged 11.5gm% and at termination, 9.5gm% in the SAH group and 11.0 gm% and 9.5gm% in the saline group. Pre-hemorrhage mean values for Pa $CO_2$, $PaO_2$ and pH were 41 ± 3.8, 128 ± 20.0 and 7.37 ± 0.56, respectively, in the SAH monkeys. Post-hemorrhage values were 39 ± 3.6, 137 ± 18.5, and 7.37 ± 0.58. $PaCO_2$, $PaO_2$ and pH mean values for the monkeys receiving saline SAS injection were not significantly different ($p > 0.05$) from the SAH group.

## B. Electroencephalographic Changes

Control monkeys demonstrated no appreciable changes in EEG over the duration of the experiments.

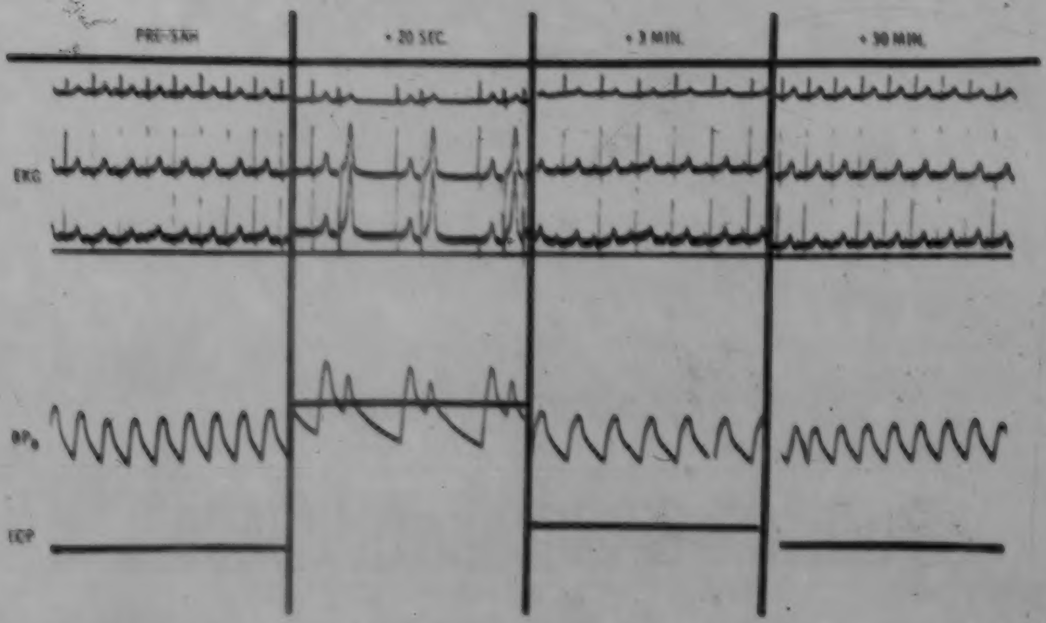


Figure 10: Cardiovascular and intracranial pressure responses to SAH.

Monkeys subjected to SAH exhibited changes begining approximately 10 seconds following the start of blood injection. The EEG became flat at 15-20 seconds and remained iso-electric for 30 to 120 seconds. Giant amplitude slow waves then appeared and lasted up to 5 minutes. Electrical activity gradually reverted towards normal although there was generally increased sporadic delta and theta activity as compared to the baseline trace. In the animals showing a progressive reduction in CBF, progressive loss of amplitude and decreased frequency was exhibited (Figure 11).

Changes in the EEG pattern following saline injection were similar to the response seen after SAH. However, unlike the frequent deterioration seen after blood injection, the electrical activity returned to normal within 5-10 minutes.

C. Cerebral Blood Flow

In general, a good correlation between flow values obtained by the stochastic and initial slope methods was found (Table 1). The mean values with standard deviation calculated by the stochastic and initial slope methods in control animals were 59 ± 16 and 66 ± 19 ml/100 gm/min., respectively. In the saline study, pre-injection mean flow values were 56 ± 11 and 59 ± 19, while in the hemorrhage study, pre-injection flows were 61 ± 14 and 65 ± 16 ml/100gm/min. Flow values calculated by the compartmental analysis were consistently lower.

The control animals demonstrated no significant variation ($p > 0.05$) in CBF throughout the experiments. Three animals were observed for 5 hours and one for 3 hours (figure 12).

Of the animals that received 4 ml's saline into the subarachnoid space (SAS), one animal showed no significant change in CBF

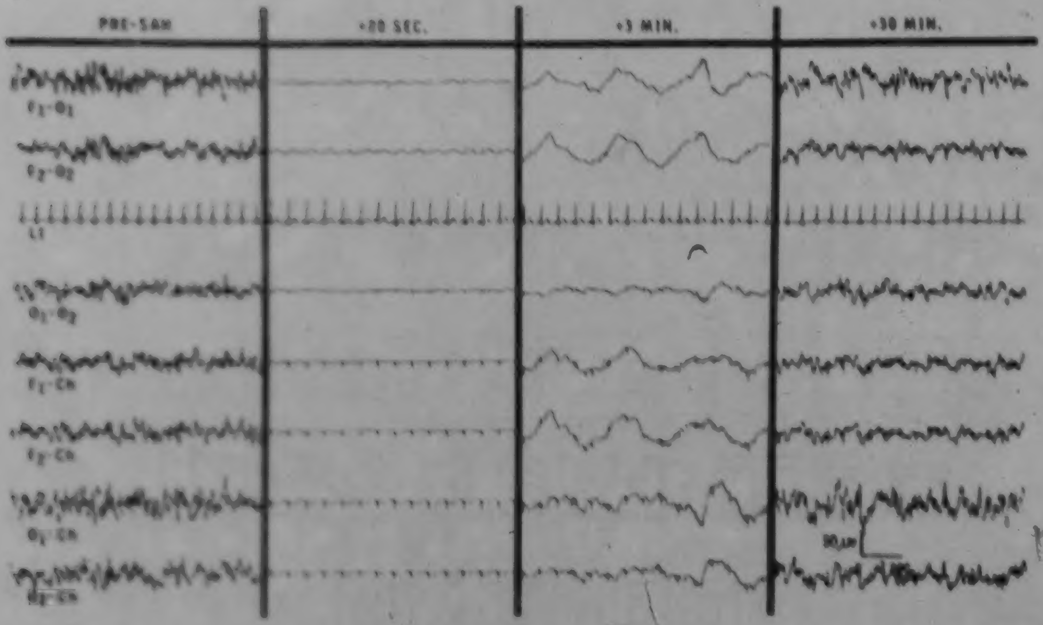


Figure 11. Electroencephalographic changes observed after SAH (15mm/sec.).

TABLE I

| | CONTROL | SALINE | | SAH | |
|---|---|---|---|---|---|
| No. Monkeys | 4 | 4 | | 14 | |
| | | PRE | POST | PRE | POST |
| Cc | 49 ± 12 | 47 ± 7 | 61 ± 9 | 50 ± 7 | *37 ± 13 |
| H/Ac | 59 ± 16 | 56 ± 11 | 76 ± 10 | 61 ± 14 | *41 ± 14 |
| ISIc | 66 ± 19 | 59 ± 19 | 77 ± 8 | 65 ± 16 | *42 ± 18 |

Mean and standard deviation of CBF Values (ml/100gm/min.) determined by Compartmental (C), Stochastic (H/A) and Initial Slope Index Methods (ISI) in 4 control monkeys. Mean pre- and post-injection values and standard deviation of 4 saline and 14 SAH monkeys.

* Uncorrected values.

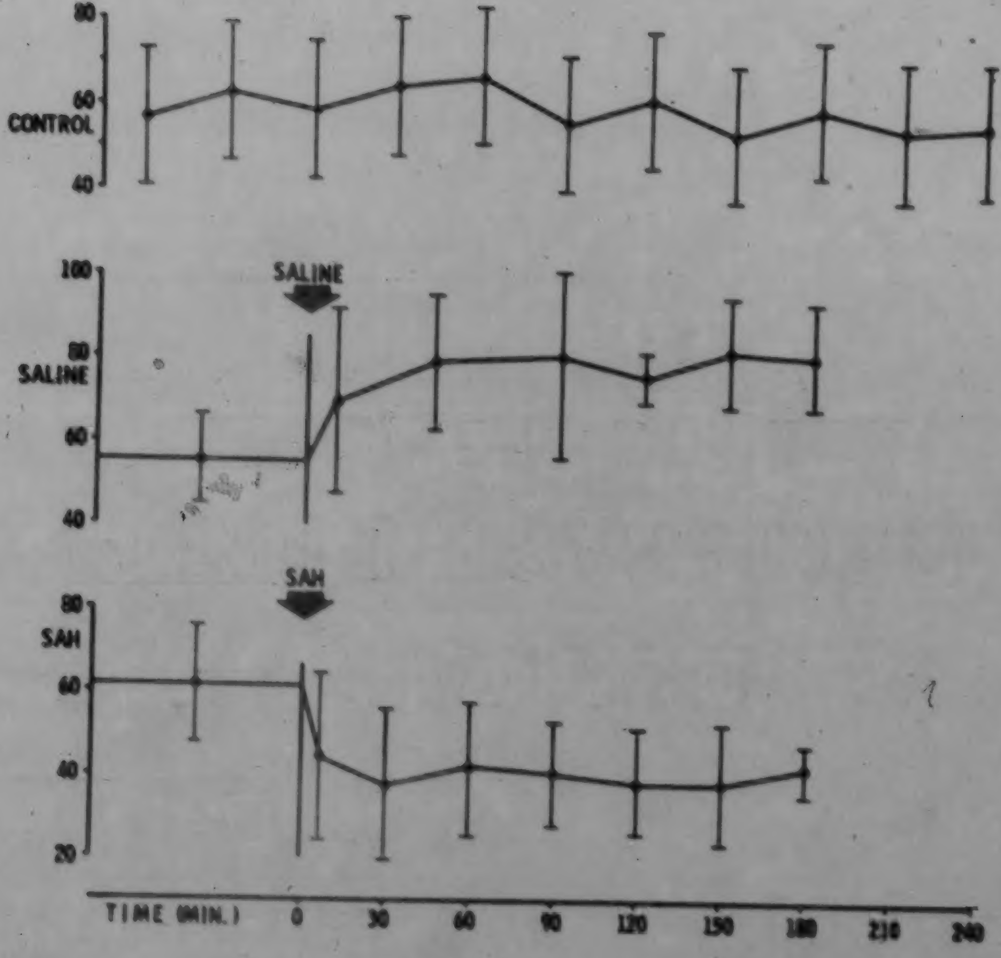


Figure 12: Mean and stardard deviation of CBF (ml/100gm/min) of four control monkeys observed over a 5-hour period. Pre- and post-injection CBF values of four monkeys receiving SAS saline and 12 monkeys subjected to SAH. Stochastic (H/A) values used.

($p > 0.05$); in another, the CBF was significantly increased for a period of 1 1/2 hours then reverted to pre-injection values. One monkey demonstrated an immediate increase in CBF for a one-hour period, returned to pre-injection values during the second hour, and finally increased significantly during the third hour of observation. The fourth animal showed increased cerebral perfusion for the 3-hour duration. Thus, in 3 of 4 monkeys, injection of acidic (pH 6.1) saline into the SAS caused an immediate increase in CBF for a period lasting an hour or more.

Of the 14 animals subjected to SAH, 12 showed a significant decrease in cerebral perfusion ($p < 0.02$). The mean pre- and post-SAH (H/A) CBF measurements were 61 ± 14 and 41 ± 14 ml/100gm/min., respectively. In 7 monkeys, the response to SAH was immediate (first post-SAH measurement), in 4 monkeys reduction was present after 30 minutes (second measurement), and in 1 animal perfusion progressively decreased after 60 minutes. Animals exhibiting an immediate decrease frequently showed lower perfusion rates but this finding was not consistent.

### D. Neurological Assessment

The control monkeys showed no impairment of consciousness or focal neurological deficit at termination of the experiments. These animals were observed for a period of approximately 2 weeks and were utilized in subsequent experiments.

The monkeys subjected to subarachnoid saline injection demonstrated no neurologic deficit following the experiments. They were sacrificed after one hour of observation to allow for pathologic examination of the brains.

Animals receiving 4 ml's of autogenous blood were observed for a period of one hour after reversal medication (atropine and neostigmine) was administered. Two animals showed no significant neurological deficit following SAH. These were arbitrarily classified as grades I and II. There was no significant changes in the mean CBF of these two animals. Eight animals were found to have definite gross neurologic deficit and all showed a significant decrease in mean CBF. However, all these animals appeared as if they would survive and their neurologic condition was improving at the time of final assessment. They were classified as grades III and IV. Four animals were moribund with failing vital signs at the time of the post-anesthetic neurologic examination (grade V) and these animals showed the lowest mean post-hemorrhage CBF values (Table II).

## II. STUDY B: THE EFFECT OF SAH AND SDH ON REGIONAL CEREBRAL BLOOD FLOW, INTRADURAL ARTERIAL CALIBER AND NEUROLOGICAL STATUS

### A. Control Series

#### (a) Cardiovascular Responses

The arterial blood pressure in the six control monkeys averaged 113 ± 9 mm Hg while the mean heart rate was 206 ± 19 beats/minute. Cardiac rhythm abnormalities were not seen in the entire series. Hemoglobin and hematocrit determinations made at the onset, mid-point and prior to termination of each experiment averaged 12.9 ± 0.5 gm%, 37.3 ± 1.9 vol%; 11.7 ± 0.9 gm%, 34.0 ± 2.2 vol%; and 10.6 ± 0.9 gm%, 30.8 ± 2.1 vol% respectively. Arterial $PCO_2$, $PO_2$ and pH values averaged 39.1 ± 3.7 mm Hg, 113.2 ± 16.9 mm Hg and 7.32 ± 0.4 respectively.

### TABLE II

| NEUROLOGICAL GRADE | NUMBER MONKEYS | MEAN PRE-SAH CBF(ml/100gm/min.) | | | MEAN POST-SAH CBF(ml/100gm/min) | | | COMPARISON BETWEEN MEAN PRE&POST SAH CBF | | | SIGNIFICANCE OF DATA ANALYSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cc | H/Ac | ISIc | C | H/A | ISI | C | H/A | ISI | |
| I & II | 2 | 44 ± 1 | 57 ± 2 | 65 ± 2 | 49 ± 3 | 61 ± 11 | 65 ± 15 | t = 1.981 | 0.403 | 0.114 | No significant change |
| | | | | | | | | p = > .15 | > .30 | > .40 | |
| III & IV | 8 | 53 ± 6 | 66 ± 11 | 74 ± 15 | 40 ± 7 | 45 ± 9 | 46 ± 10 | t = 3.491 | 4.149 | 4.091 | Significant decrease |
| | | | | | | | | p = < .005 | < .005 | < .005 | |
| V | 4 | 43 ± 4 | 48 ± 6 | 49 ± 5 | 23 ± 12 | 23 ± 15 | 22 ± 13 | t = 2.163 | 2.499 | 3.422 | Significant decrease |
| | | | | | | | | p = < .10 | < .05 | < .025 | |

Correlation between clinical grading of monkeys and cerebral blood flow (ml/100gm/min). Mean pre and post hemorrhage flow values with standard deviation calculated by compartmental (C), stochastic (H/A) and Initial-slope-index (ISI) methods. t-test comparison of mean pre and post-hemorrhage flow values are included.

### (b) Regional Cerebral Blood Flow (rCBF)

Reproducibility of the method employed to measure CBF was examined in a monkey by performing flow determinations in the same animal on two different occasions one week apart. CBF values determined by the compartmental, stochastic and initial-slope-index methods averaged 52 ± 3, 43 ± 3 and 43 ± 3 ml/100gm/min in the first experiment compared to values of 53 ± 2, 44 ± 4 and 48 ± 5 ml/100gm/min in the second study. This study suggested that the technique utilized for CBF measurement was reproducible.

In six control monkeys, rCBF determined at intervals of 30-45 minutes for a period of 5-6 hours, no significant variation in cerebral perfusion occurred throughout this time period (Figure 13). The mean compartmental, stochastic and initial-slope-index rCBF values and standard deviations are shown in Figure 14. Flow values recorded from the frontal, central, parietal and temporal areas were not significantly different from each other. Mean rCBF values in the cerebellum (detector 6) was 25% lower than the combined means of flows in the cerebral hemisphere (detectors 1,2,3,and 5), while orbito-maxillary tissue flow (detector 4) was reduced by approximately 50%.

The mean CBF measured by the contralateral single detector system i.e.(pHBF), was not significantly different from the mean sum of the ipsilateral multidetector system (detectors 1, 2, 3, and 5). The small variations present were attributable to extracerebral contamination and counting geometry differences.

### (c) Angiography

Lateral angiograms performed at the onset and upon termination of each experiment did not reveal significant change in vessel caliber.

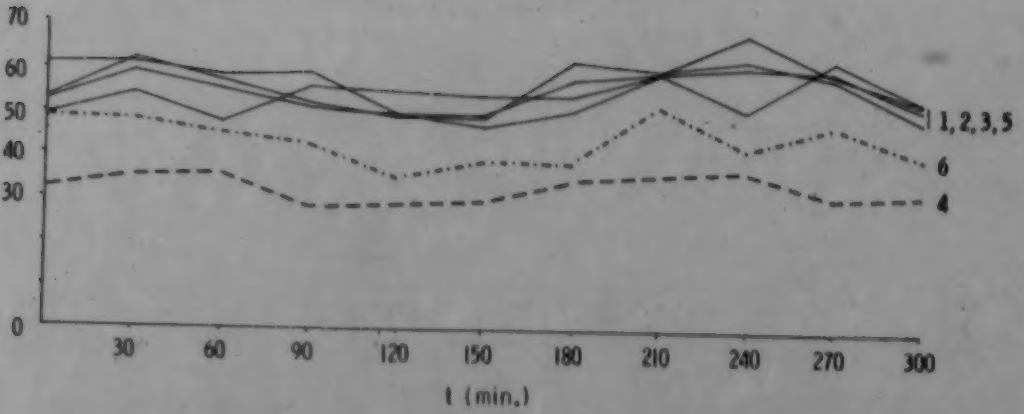


Figure 13: Mean stochastic rCBF values (ml/100gm/min) in six control monkeys measured over a 5-hour period. Cerebral values (detectors 1, 2, 3 & 5) show little inter-regional variation. Mean cerebellar flow (detector 6) is approximately 25% below and mean orbital tissue perfusion (detector 4) is 50% lower than mean hemispheric values.

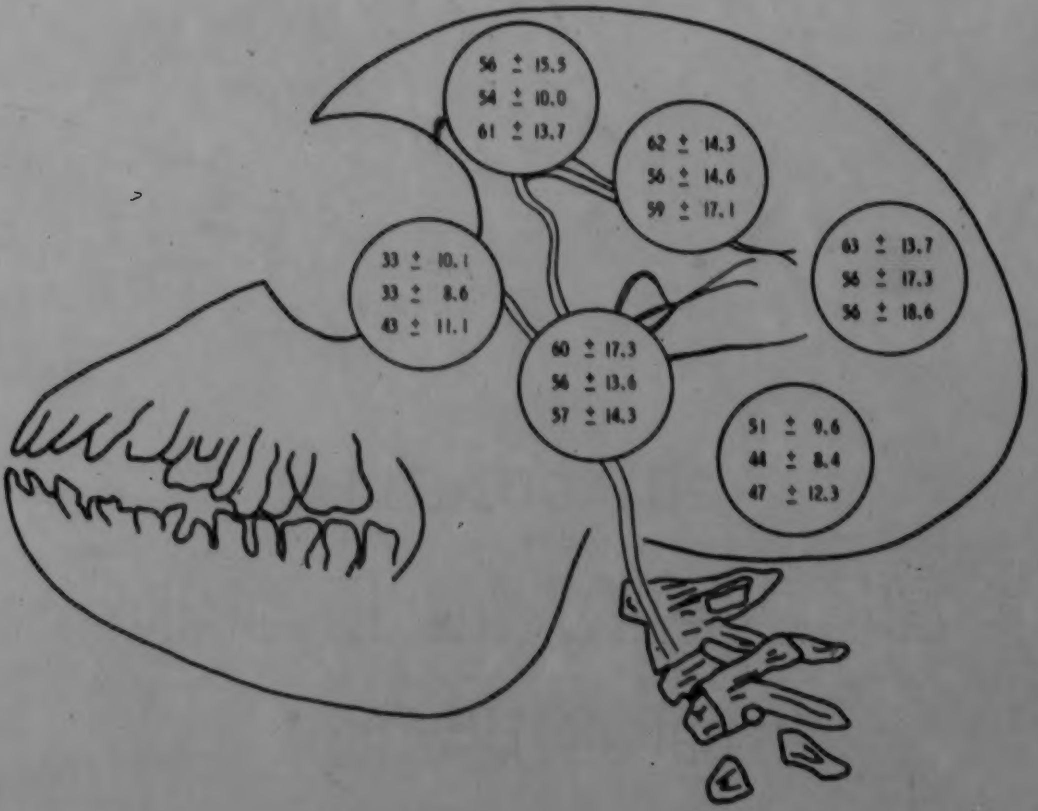


Figure 14: Mean rCBF and standard deviations (ml/100gm/min) determined by the Compartmental (top values), Stochastic (middle values) and Initial-slope-index (bottom values) methods over a 5-hour period in six control monkeys.

### (d) Neurological Assessment

Using the five-division grading system outlined previously, all control animals were classified as grade I and were utilized for subsequent experiments.

## B. Subarachnoid Hemorrhage Series

### (a) Cardiovascular and Intracranial Pressure Responses

The mean arterial BP prior to SAH in eight monkeys studied was 114 ± 12 mm Hg while the post-SAH values averaged 117 ± 17 mm Hg. Injection of 4 ml autogenous blood into the basal cisterns produced systemic hypertension beginning at 5-10 seconds and reaching a maximal value (173 ± 14 mm Hg) within 30-45 seconds after the onset of injections. Within 2-8 minutes following SAH, systemic BP returned to baseline levels.

Pre-hemorrhage heart rate (HR) averaged 209 ± 17, compared to 181 ± 30 beats/minute in the post-SAH period. Bradycardia was usually observed within 30 seconds after onset of injection and the minimal value averaged 121 ± 25 beats/minute. Post-injection values beyond the first 5-10 minutes were not significantly different from pre-SAH values.

Cardiac arrythmias, which were first observed 25-45 seconds from the onset of SAH, were seldom present after five minutes. The most frequent changes observed included sinus arrythmia, nodal beats, nodal rhythm and premature ventricular contractions. Other EKG abnormalities, i.e., increased T and P wave amplitude, S-T elevation, U-waves, were also common.

Intracranial pressure changes during and after SAH were monitored in three animals. Pre-hemorrhage values averaged 17 ± 5 mm

Hg compared with 34 ± 11 mm Hg in the post-SAH period. After a mean peak pressure of 140 ± 8 mm Hg, observed at 20-30 seconds, ICP returned to near baseline values within 5 minutes. Following this initial rapid decrease, ICP values gradually increased during the subsequent 3-hour period, perhaps secondary to ensuing cerebral edema.

Mean pre-hemorrhage $PaCO_2$ and $PaO_2$ and pH values were 38.9 ± 3.3 mm Hg, 110 ± 13.5 mm Hg and 7.37 ± 0.1, respectively. Post-SAH values averaged 39.0 ± 3.0 mm Hg, 110 ± 9.5 mm Hg, and 7.37 ± 0.1. Hemoglobin and hematocrit values at the onset of the experiments averaged 12.5 ± 1.5 gm%, 37.0 ± 5.2 vol%, prior to SAH, 11.5 ± 1.7 gm%, 33.4 ± 5.2 vol%, and upon termination, 10.5 ± 1.8 gm% and 30.3 ± 4.9 vol%.

(b) Regional Cerebral Blood Flow (rCBF)

Of the eight monkeys subject to SAH, six (75%) showed a significant reduction in rCBF (Figure 15). The decrease was present in all brain regions viewed by the 5-ipsilateral detectors. Orbito-maxillary tissue perfusion (detector 4) was not significantly affected by SAH. The reduction in rCBF was immediate (first rCBF at 3 minutes post-SAH) and flow values remained significantly reduced for the 3-hour duration. Little variation in rCBF occurred after reduced perfusion was established. Contralateral hemispheric perfusion measured by the single detector system was also significantly decreased. Mean pre- and post-SAH rCBF values with standard deviations calculated by the compartmental, stochastic and initial-slope-index methods are represented in Figure 16. One animal subjected to rCBF studies 24 hours after SAH continued to show a significant global reduction in cerebral perfusion.

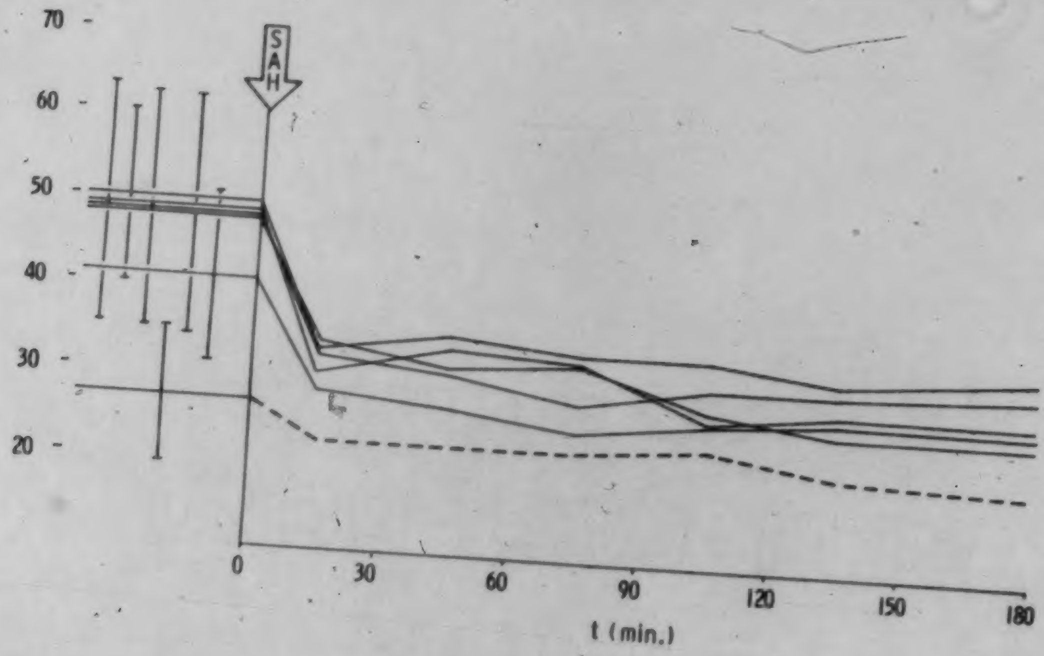


Figure 15: Decrease in rCBF (ml/100gm/min) in six of eight monkeys subjected to SAH. Cerebral and cerebellar perfusion decreased after SAH, whereas orbito-maxillary tissue flow (dotted line) remained constant. Stochastic (H/A) values used.

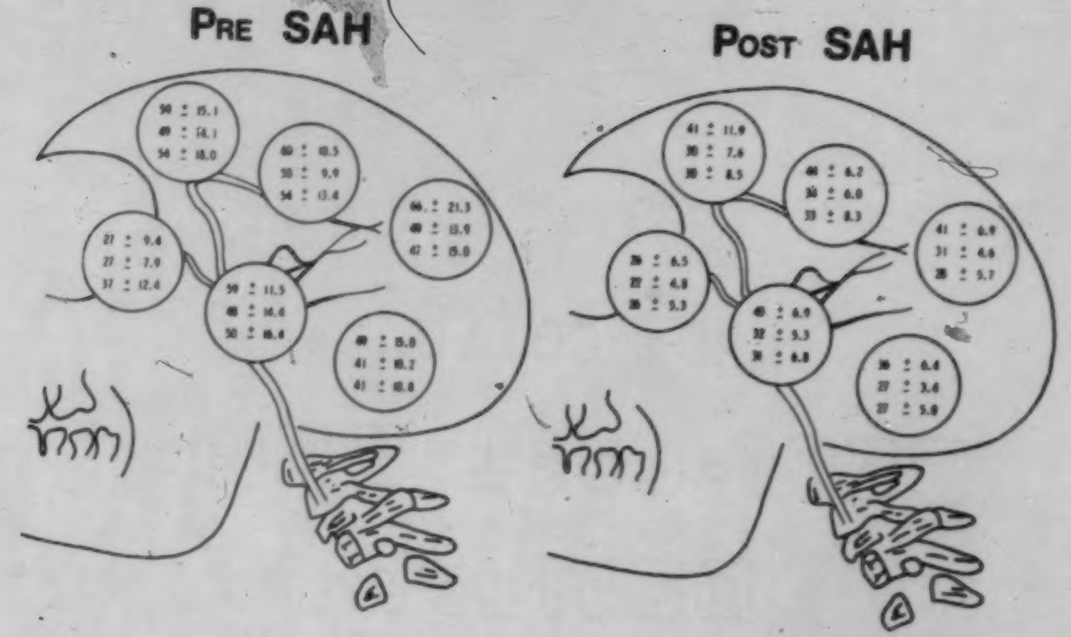


Figure 16: Mean pre- and post-SAH Compartmental, Stochastic (H/A) and Initial-slope-index rCBF values (ml/100gm/min) in six monkeys which displayed a significant decrease in cerebral perfusion.

Two monkeys (25%) with profuse SAH did not develop a decrease in rCBF in the 3-hour period following injection (Figure 17).

(c) Angiography

Baseline lateral angiograms were performed immediately following internal carotid artery catheterization and after needle insertion into the SAS. Needle placement did not produce alteration in vessel diameter ($p>0.05$). Post-hemorrhage angiography was carried out immediately after measurement of the first post-SAH rCBF (i.e., at 20 minutes post-SAH), 1.5 hours and 3 hours post-hemorrhage. In one study, angiography was carried out 24 hours after induction of SAH.

Significant generalized vasospasm of the intradural vessels was present in all monkeys subjected to SAH (Figure 18). The mean percentage reduction in all vessels measured at the predetermined post-SAH times was 30 ± 9.3%.

Table III compares the mean percentage decrease of the intradural arterial tree in monkeys which showed a significant rCBF decreased compared with the animals which did not. Monkeys not displaying reduction in rCBF exhibited a mean vessel caliber decrease of 26 ± 9.8%. Although spasm was intense initially, there was tendency for some vessel relaxation, although the increase in vessel size was not statistically significant (Figure 19).

(d) Neurological Assessment

The animals with significant reduction in cerebral blood flow following SAH displayed severe neurologic abnormalities. All were severely obtunded, hemiplegic or quadriplegic and responded poorly to all forms of stimuli (Grade IV.). Occasionally, intermittent generalized clonic seizures were present. One animal developed failing vital signs

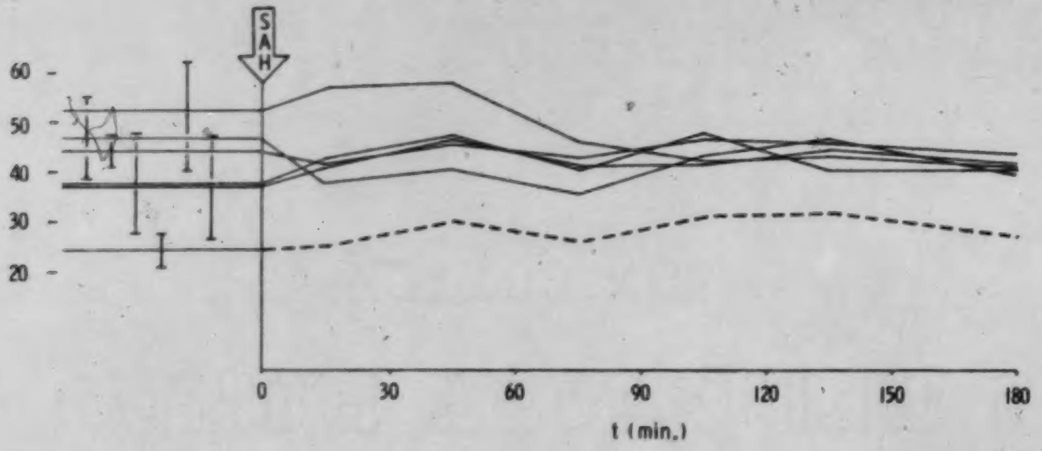


Figure 17: Mean pre- and post-SAH rCBF values (ml/100gm/min) in monkeys not exhibiting a decrease in cerebral perfusion. Stochastic values used.

Figure 18: Serial lateral angiograms of a monkey: A. pre-SAH; B. 20 minutes post-SAH and C. three hours post SAH. Diffuse intradural vasospasm is observed in post-SAH films B and C. CBF was significantly reduced post-SAH.

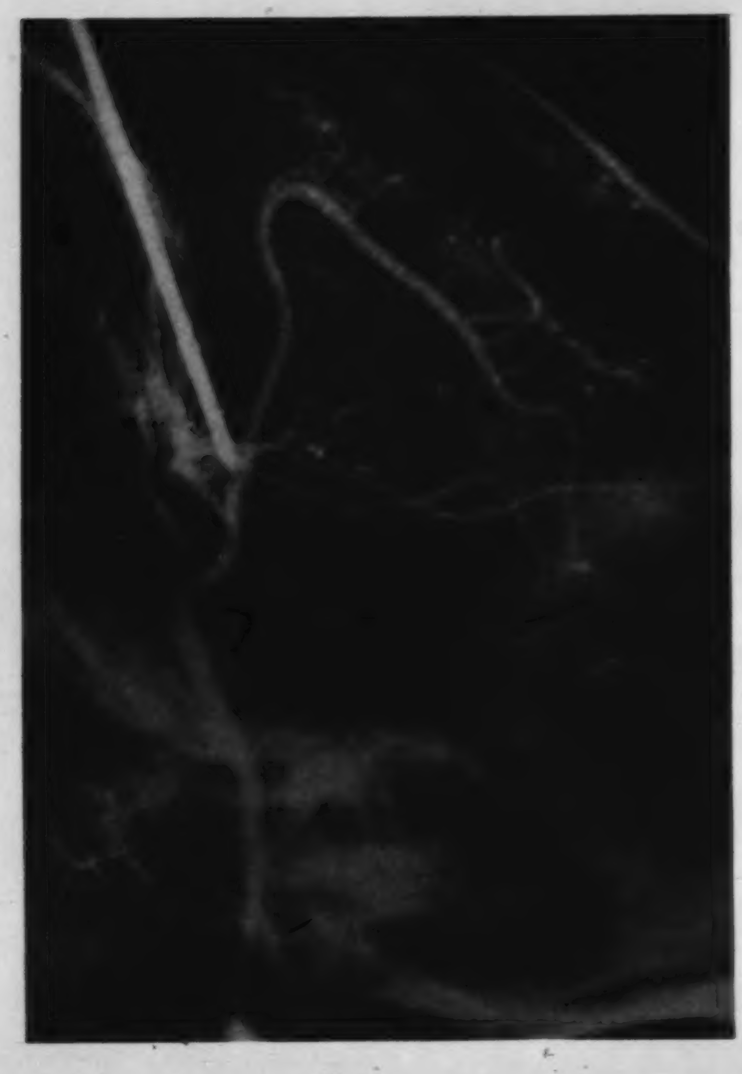


Figure 18A:

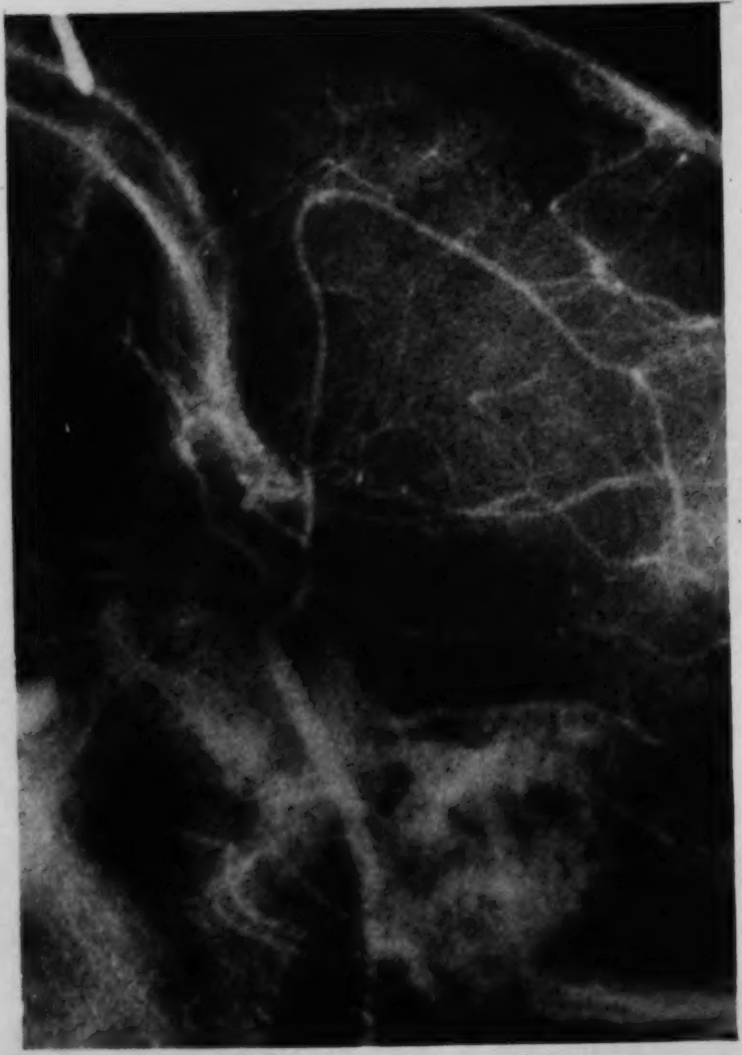


Figure 18B:

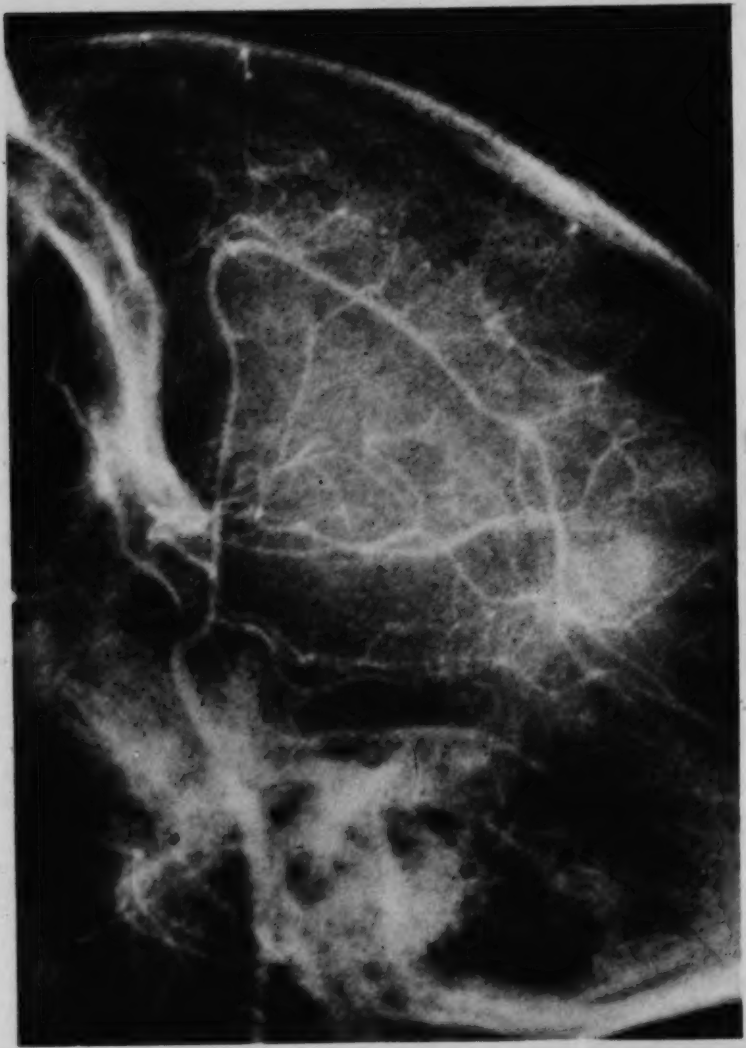


Figure 18C:

## TABLE III.

## POST-SAH PERCENT VESSEL CALIBER CONSTRICTION IN MONKEYS SHOWING A SIGNIFICANT AND NON-SIGNIFICANT rCBF DECREASE.

| | POST-SAH PERCENT VESSEL CALIBER DECREASE | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vessel Measured | ICA (Ophth.) | | | IICA | | | PPA | | | DPA | | | MCA | | |
| | Time (min) | 20 | 90 | 180 | 20 | 90 | 180 | 20 | 90 | 180 | 20 | 90 | 180 | 20 | 90 | 180 |
| SIGNIFICANT rCBF REDUCTION | Mean % Decrease | 25 | 31 | 29 | 25 | 33 | 25 | 37 | 33 | 30 | 53 | 42 | 35 | 36 | 30 | 32 |
| | S.d. | 11.5 | 10.6 | 4.4 | 10.4 | 3.8 | 12.6 | 13.6 | 13.4 | 13.9 | 25.4 | 8.2 | 4.0 | 7.5 | 12.8 | 12.6 |
| NO rCBF REDUCTION | Mean % Decrease | 26 | 19 | 15 | 45 | 34 | 25 | 34 | 18 | 21 | 46 | 19 | 20 | 26 | 22 | 16 |
| | S.d. | 0.6 | 7.6 | 9.1 | 13.0 | 16.7 | 0.3 | 10.7 | 3.3 | 0.6 | 28.2 | 24.9 | 13.9 | 6.8 | 7.4 | 9.5 |

ICA (Ophth.) = Internal Carotid Artery at Ophthalmic Artery origin; IICA = Intradural Internal Carotid Artery;
PPA = Proximal Pericallosal Artery; DPA = Distal Pericallosal Artery; MCA = Middle Cerebral Artery.

Figure 19: Lateral angiograms taken A. prior to SAH; B. 20 minutes post-SAH and C. 3 hours post-SAH. Although no decrease in rCBF occurred after SAH, diffuse intradural vasospasm was exhibited in post-SAH films (B and C).

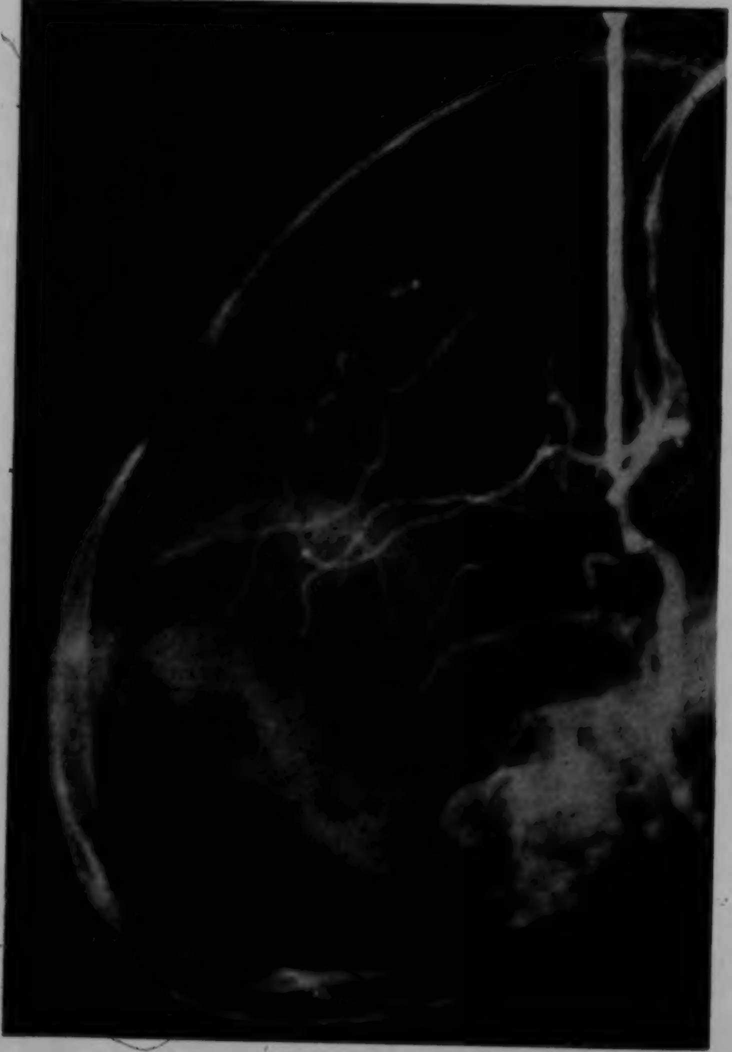


Figure 19A:

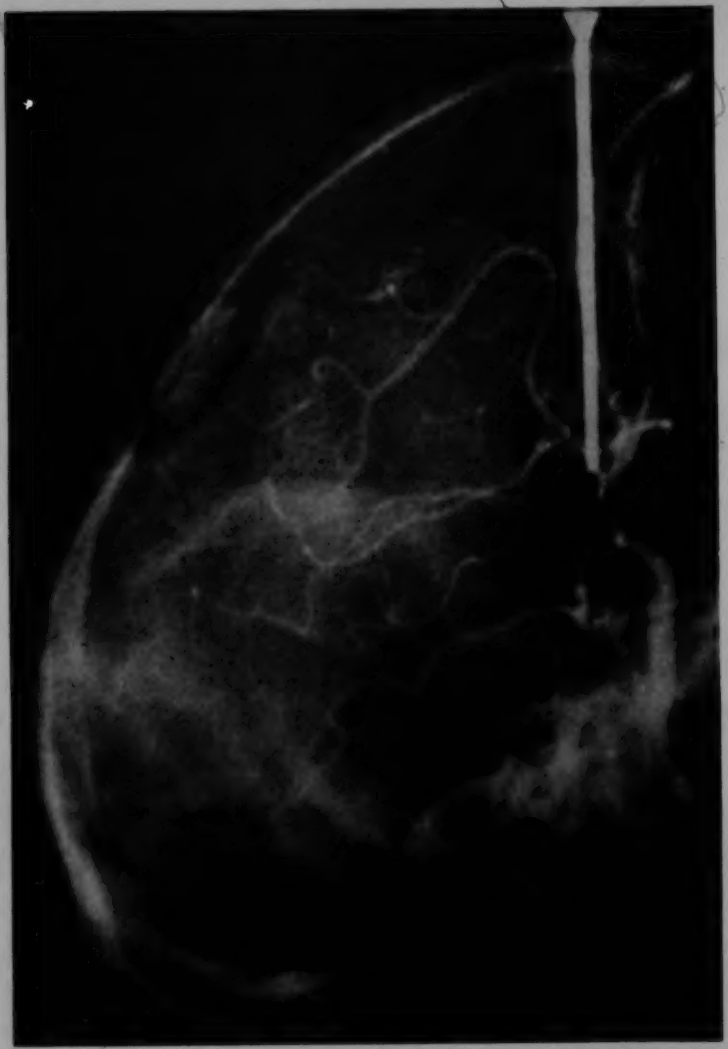


Figure 19B:

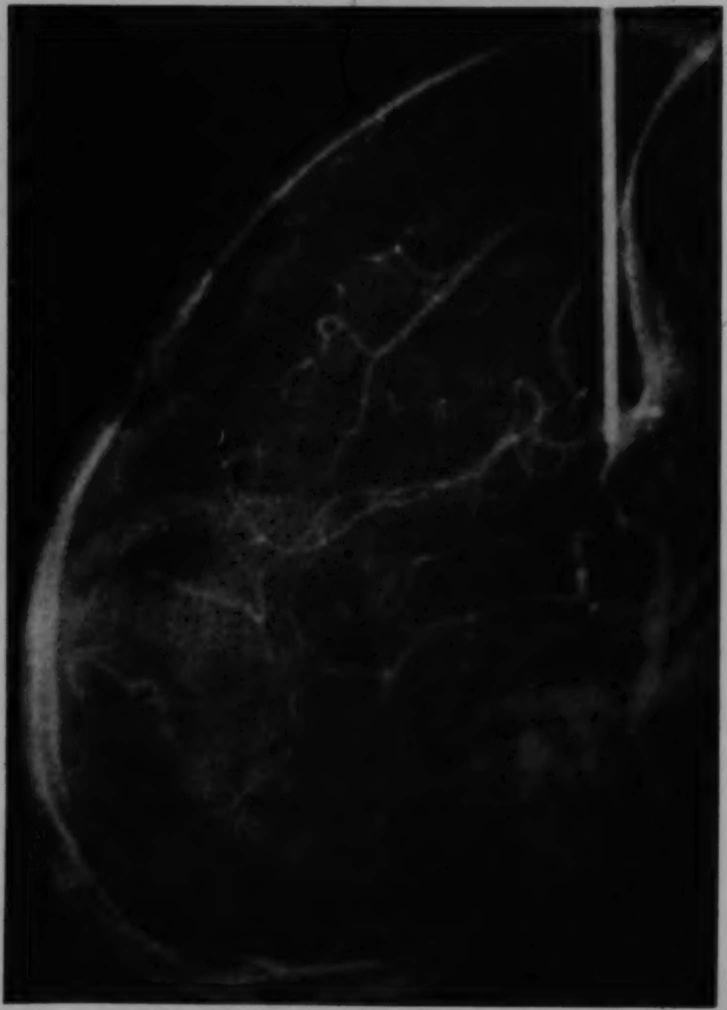


Figure 19C:

during the period of examination and died (Grade V).

Monkeys exhibiting vasospasm but no reduction in cerebral perfusion after SAH appeared mildly obtunded but did not display a neurologic deficit. They were classified as Grade II clinically.

### C. Subdural Hemorrhage Series

Seven monkeys, grouped into three categories by virtue of the amount of blood present in the subdural space on post-mortem examination, comprised the subdural hemorrhage series. Two animals only displaying subdural hemorrhage were classified as Group A; three showed primarily SDH with a minute amount of blood in the subarachnoid space -- Group B; and two animals presented with 20-30 percent SAH component -- Group C.

#### (a) Cardiovascular and Intracranial Pressure Responses

There were no significant differences in cardiovascular and intracranial pressure responses between the three groups of monkeys studied. The mean arterial BP prior to induction of SDH was 108 ± 19 mm Hg while the post-SDH values averaged 118 ± 24 mm Hg. subdural hemorrhage produced a systemic hypertension beginning at about 10 seconds and reached a mean peak value (169 ± 25 mm Hg) within 60 seconds from onset of injection. Post-hemorrhage values generally reverted to baseline values in 5-10 minutes.

The mean baseline heart rate (HR), 197 ± 30 beats/minute, was not significantly different from mean post-hemorrhage value (178 ± 39 beats/minute). Decrease in HR was observed at 5-15 seconds after onset of injection and the mean minimal value (114 ± 46 beats/minute) was reached at 20-30 seconds. Post-hemorrhage HR beyond 25 minutes was not significantly different from baseline values.

Arrythmias were frequently observed in this series and usually began 20-30 seconds after SDH. The EKG changes were similar to those observed after SAH.

Pre-hemorrhage intracranial pressure averaged 20 ± 8 mm Hg compared with 45 ± 20 mm Hg in the 3-hour period after SDH. The mean peak ICP observed at 30-50 seconds post-injection was 150 ± 24 mm Hg. Unlike the SAH series where ICP usually reverted to baseline levels within 5 minutes, the pressure gradually decreased but did not reach baseline levels in the entire 3-hour period.

Pre-SDH $PaCO_2$, $PaO_2$ and pH values were 40.1 ± 3.9 mm Hg, 109 ± 13.4 ~~mm Hg~~ and 7.35 ± 0.04 compared with post SDH of 39.6 ± 4.5 mm Hg, 110 ± 11.1 mm Hg 7.35 ± 0.06.

(a) Regional Cerebral Blood Flow (rCBF)

(i) Group A (SDH)

Monkeys exhibiting subdural hemorrhage without a subarachnoid component did not have a reduction in CBF over the 3-hour post-injection period. Mean pre-hemorrhage stochastic values were 57 ± 10, compared with 55 ± 14 ml/100gm/min in the post-SDH period.

(ii) Group B (SDH with a small SAH component)

These animals showed a small non-significant reduction in CBF in the post-hemorrhage period. Mean pre- and post-hemorrhage values were 52 ± 10 and 50 ± 14 ml/100m/min respectively ($p>0.05$).

(iii) Group C (SDH with a 20-30% SAH component)

In animals which displayed a 20-30 percent sub-archnoid component, a significant reduction in cerebral perfusion occurred ($p<0.02$)

(c) Angiography

(i) Group A Monkeys.

Reduced vessel caliber was observed only in arteries in the immediate vicinity of the basal subdural hemorrhage (intracavernous internal carotid and the internal carotid artery at the origin of the ophthalmic artery). There was no evidence of caliber reduction inthe intradural portion of the arterial tree.

(ii) Group B Monkeys

Decrease in arterial vessel caliber was present primarily in the intracavernous internal carotid artery, internal carotid at the ophthalmic origin and to a lesser extent the proximal segment of the middle cerebral and intra-dural internal carotid arteries. No significant reduction in the proximal and distal pericallosal artery was evident.

(iii) Group C Monkeys

The intradural arteries measured in animals which diplayed a 20-30 percent subarachnoid component showed a significant reduction in vessel caliber of 20-25 percent. Unlike Group A and B monkeys, no significant reduction in extradural vessel caliber occurred.

(d) Neurological Assessment

Monkeys subjected to subdural hemorrhage with or without a small subarachnoid component (Group A and B) appeared mildly obtunded but did not exhibit significant neurologic deficit and were classified as Grade II.

Monkeys with a 20-30 percent subarchnoid component were moderately obtunded and displayed significant neurologic abnormalities including hemiparesis, depressed response to stimuli and cranial nerve palsy. They were classified as Grade III (Table IV).

## III. STUDY C: THE EFFECT OF GRADED HYPOCAPNIA AND HYPERCAPNIA ON rCBF, INTRADURAL VESSEL REACTIVITY, AND NEUROLOGICAL STATUS IN CONTROL MONKEYS AND MONKEYS SUBJECTED TO SAH AND TSICA

### A. Control Series

#### (a) Physiological Responses

The effects of hypocapnia, normocapnia and hypercapnia on pH, $PaO_2$, HR, MBP, and ICP in nine control monkeys are presented in Table V. The mean and standard deviation of $PaCO_2$ during hypocapnia, normocapnia and hypercapnia were 23.5 ± 5.5 mm Hg, 39.8 ± 2.4 mm Hg and 61.9 ± 10.3 mm Hg respectively. Changes in $PaCO_2$ significantly ($p < 0.01$) altered arterial pH values (respiratory alkalosis and acidosis) but produced little change in $PaO_2$. Heart rate and mean blood pressure were not significantly altered ($p > 0.05$) during induced hypocapnia and hypercapnia. Stability of HR and MBP was in part due to the small step-wise differences in $PaCO_2$ and to the time alloted for the cardiovascular responses to normalize. Hypercapnia produced an increase in ICP (increase in cerebral blood volume) whereas hypocapnia caused a slight decrease. Significant changes in EKG were not observed even at extreme $PaCO_2$ levels.

#### (b) Relationship Between CBF and $PaCO_2$

A total of eighty-four CBF studies were performed in the $PaCO_2$ range of 16 mm Hg to 85 mm in nine control animals. Fifteen studies were carried out during hypocapnia, thirty-five during normocapnia (40 ± 5 mm Hg) and thirty-three during hypercapnia.

TABLE IV.

CEREBRAL BLOOD FLOW, ANGIOGRAPHY AND NEUROLOGICAL GRADE CORRELATION OF CONTROL AND MONKEYS SUBJECTED TO SUBARACHNOID AND SUBDURAL HEMORRHAGE.

| GROUP | NUMBER | rCBF | ANGIOGRAPHY | NEUROLOGICAL GRADE |
|---|---|---|---|---|
| CONTROL | 6 | No Change | No Spasm | I |
| SAH | 6 | Marked Decrease | Marked Spasm | IV-V |
| SAH | 2 | No Change | Marked Spasm | II |
| SDH | 2 | No Change | Local Spasm | II |
| SDH (small SAH component) | 3 | Mild Decrease | Local Spasm | II |
| SDH (20-30% SAH component) | 2 | Moderate Decrease | Moderate Spasm | III |

# TABLE V.

## PHYSIOLOGIC RESPONSES TO HYPOCAPNIA AND HYPERCAPNIA IN CONTROL MONKEYS

| | Hypocapnia<br>n = 15 | Normocapnia<br>n = 35 | Hypercapnia<br>n = 33 |
|---|---|---|---|
| PaCO2 (mm Hg) | 23.5 ± 5.5 | 39.8 ± 2.4 | 61.9 ± 10.3 |
| pH | 7.51 ± 0.08 | 7.35 ± 0.05 | 7.20 ± 0.08 |
| PaO2 (mm Hg) | 118 ± 13 | 122 ± 18 | 122 ± 23 |
| HR (beats/min) | 180 ± 31 | 182 ± 26 | 180 ± 19 |
| MBP (mm Hg) | 124 ± 18 | 118 ± 17 | 118 ± 21 |
| ICP (mm Hg) | 12 ± 9 | 15 ± 6 | 26 ± 12 |

Considerable variation in CBF response to $PaCO_2$ change was found amoung different animals and in individual animals (Figure 20). Cerebral perfusion increased linearly between $PaCO_2$ values of 30 mm Hg and 60 mm Hg and in this range CBF sensitivity was greatest. Cerebral blood flow response to $PaCO_2$ diminished considerably below 30 mm Hg and values below 25 mm Hg were frequently associated with a small increase in CBF. Response to $PaCO_2$ values above 65-75 mm Hg was attenuated and no further increase in cerebral perfusion occurred above a $PaCO_2$ value of 80 mm Hg.

Linear regression analysis for CBF was carried out for each of the several calculated flow values in $PaCO_2$ range of 10-80 mm Hg and these results are shown in Figure 21. An excellent correlation between the stochastic (H/A) and initial-slope-index (ISI) methods for CBF calculation was found and these two methods of calculation have been used for purposes of statistical analysis. As shown in Figure 21, grey matter flow ($F_g$) was more sensitive to $PaCO_2$ change than white matter flow ($F_w$).

Mean stochastic and initial-slope-index rCBF, mHBF and pHBF values during hypocapnia, normocapnia and hypercapnia are given in Table VI. Induced hypocapnia, to a mean $PaCO_2$ value of 23.5 ± 5.5 mm Hg from the mean normocapnic value (39.8 ± 2.4 mm Hg), caused a 40 percent decrease in mHBF, whereas hypercapnia (mean 61.9 ± 10.3 mm Hg) produced a 74 percent increase. During normocapnia, rCBF did not vary significantly ($p > 0.05$) from one cerebral area to another although flow in the central area (Probe 2) appeared to be consistently higher (Figure 22). During normocapnia, flows in the cerebellum (Probe 6) and in orbito-maxillary tissue (Probe 4) were lower than cerebral tissue by

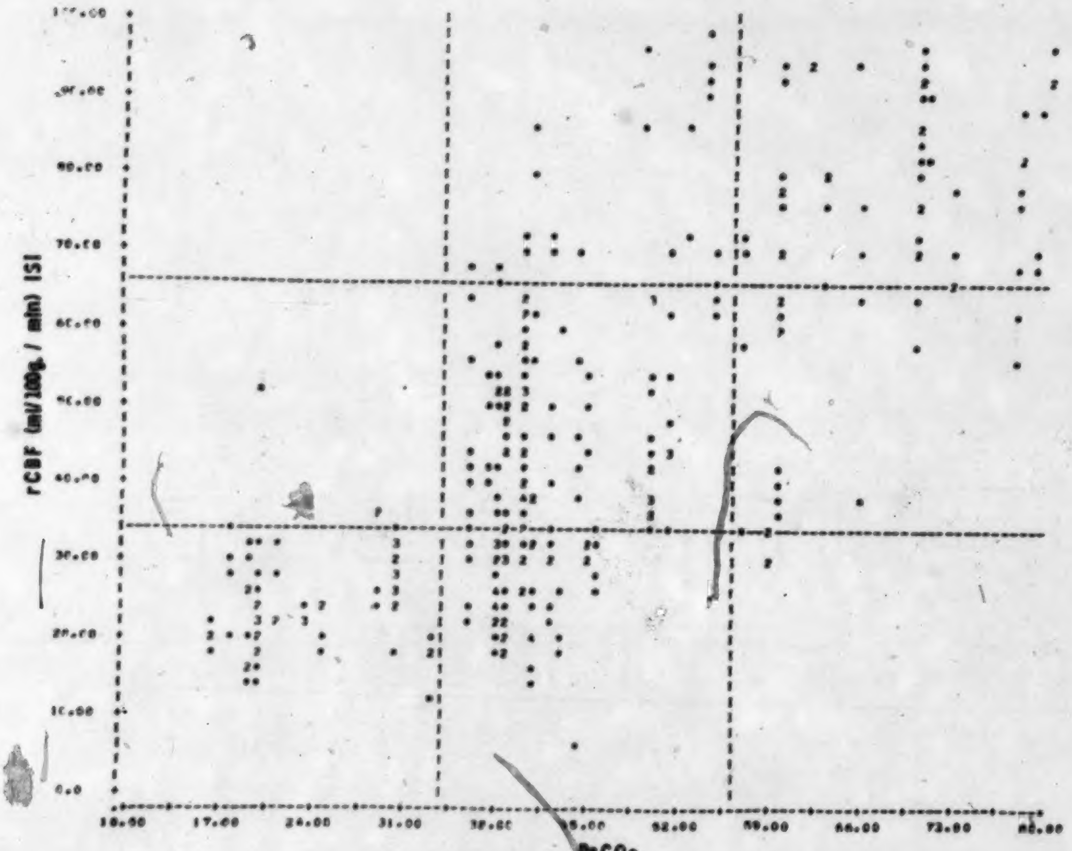


Figure 20: Computer printout showing the relationship between CBF (Initial-slope-index method) and PaCO2 change in nine control animals (PaCO2 range 10-80mmHg).

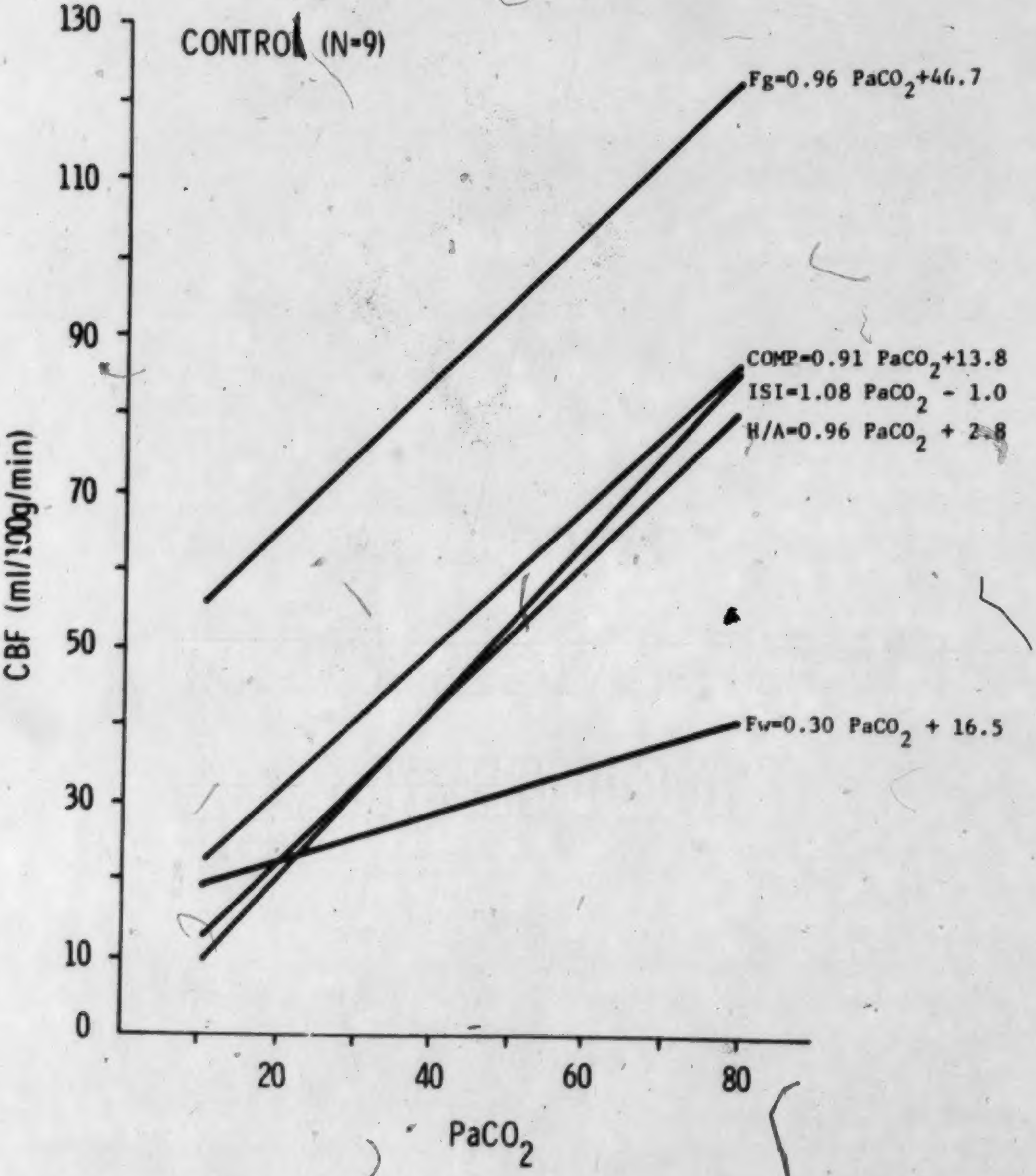


Figure 21: Linear regression analysis for Fg, Fw, FC, FH/A and FISI in the PaCO2 range 10-80mmHg (control monkeys). Equations describing the relationship of CBF to PaCO2 are included.

## TABLE VI.

REGIONAL CEREBRAL (rCBF), MEAN HEMISPHERIC (mHBF) AND PARTIAL HEMISPHERIC (pHBF) BLOOD FLOW RESPONSES (H/A AND ISI ANALYSIS) to PaCO2 CHANGE IN CONTROL ANIMALS

| | Hypocapnia | | Normocapnia | | Hypercapnia | |
|---|---|---|---|---|---|---|
| | PaCO2 (23.5 ± 5.5) | | PaCO2 (39.8 ± 2.4) | | PaCO2 (61.9 ± 10.3) | |
| | H/A | ISI | H/A | ISI | H/A | ISI |
| rCBF | | | | | | |
| P1 | [illegible] ± 7 | 23 ± 5 | 38 ± 17 | 41 ± 21 | 65 ± 23 | 77 ± 25 |
| P2 | 26 ± 6 | 26 ± 6 | 41 ± 16 | 46 ± 19 | 65 ± 21 | 76 ± 23 |
| P3 | 25 ± 4 | 24 ± 5 | 40 ± 13 | 39 ± 12 | 65 ± 22 | 72 ± 27 |
| P4 | 19 ± 4 | 25 ± 8 | 22 ± 6 | 30 ± 8 | 40 ± 18 | 52 ± 17 |
| P5 | 27 ± 6 | 28 ± 9 | 38 ± 12 | 38 ± 14 | 74 ± 21 | 78 ± 23 |
| P6 | 22 ± 5 | 24 ± 6 | 30 ± 9 | 33 ± 11 | 48 ± 18 | 55 ± 21 |
| mHBF | 26 ± 6 | 25 ± 6 | 39 ± 15 | 41 ± 17 | 68 ± 22 | 76 ± 26 |
| pHBF | 23 ± 5 | 23.5 ± 4.4 | 35 ± 13 | 38 ± 16 | 65 ± 21 | 76 ± 25 |

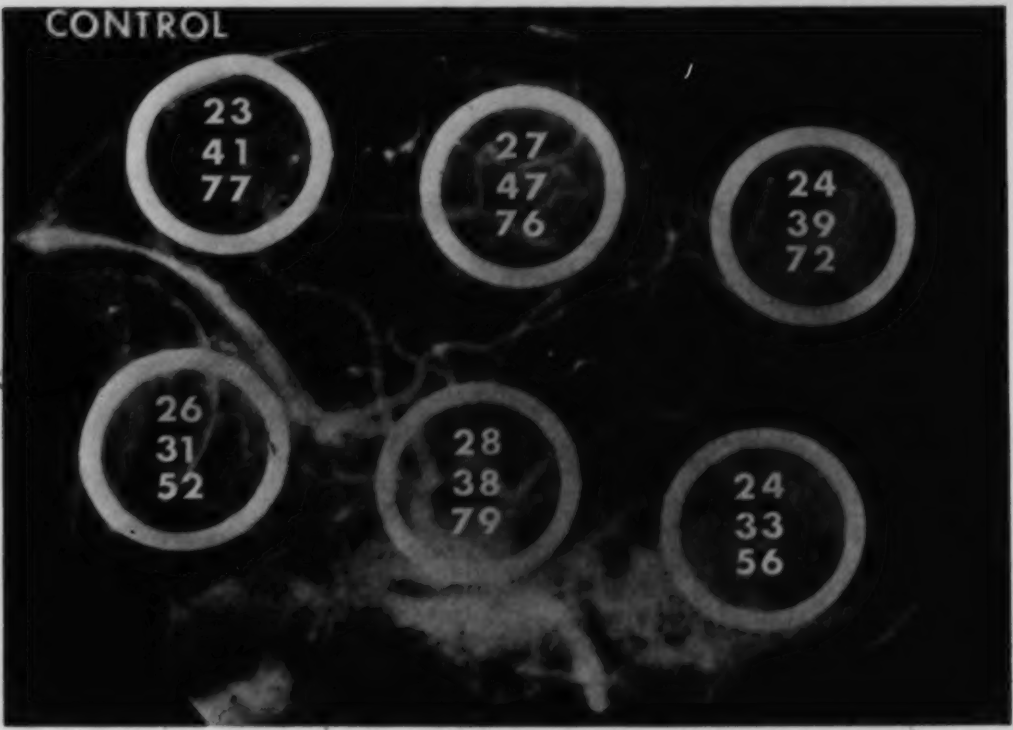


Figure 22: Effect of hypocapnia and hypercapnia on mean cerebral (detectors 1, 2, 3 and 5), cerebellar (detector 6) and orbito-maxillary (detector 4) tissue flows (ISI analysis) in nine control animals. Top values - flows during hypocapnia; middle values - flows during normocapnia; bottom values - flows during hypercapnia (ml/100gm/min).

20 and 35 percent respectively. Cerebral tissue flows were more influenced by $PaCO_2$ change than were the cerebellar or orbital tissues, thus suggesting a greater responsiveness.

Mean hemispheric blood flow and vessel caliber resonses to severe hypocapnia and hypercapnia in individual control animals are shown in Table VII. Six animals were made hypocapnic and all exhibited a decrease in mHBF. Hypercapnia was induced in eight animals and all showed a marked increase in mHBF.

(c) Vessel Diameter Responses to $PaCO_2$ Change

To test vessel reactivity to $PaCO_2$ change, angiographic studies were performed in nine animals. Angiograms were obtained in five animals during hypocapnia, in nine during normocapnia and in seven during hypercapnia. Vessel caliber measurements were made at predetermined, fixed locations on the arteries (Figure 23).

The effect of contrast material on vessel diameter during each angiographic sitting (constant MBP and $PaCO_2$) in control and experimental animals was analyzed by measuring the change in vessel caliber with each successive Conray injection. Although a small increase in vessel caliber between the first and subsequent Conray injections was frequently present, the induced vasodilation did not reach levels of statistical significance ($p>0.05$)

Of the five monkeys subjected to severe hypocapnia, two exhibited a marked reduction in vessel diameter, while two others displayed mild constriction. In one animal (Monkey #3), vasoconstriction was absent (Table VII). In general, CBF studies performed just prior to angiography correlated well with the angiographic findings.

Correlative CBF and angiographic studies were performed in

# TABLE VII.

## MEAN HEMISPHERIC BLOOD FLOW (H/A AND ISI ANALYSIS) AND VESSEL CALIBER (mm) RESPONSES TO SEVERE HYPOCAPNIA AND HYPERCAPNIA IN INDIVIDUAL CONTROL MONKEYS

| | Hypocapnia | | | | | | | Normocapnia | | | | | | | Hypercapnia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M# | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA |
| 1 | 20 | **<br>14.5<br>±3.6 | **<br>18.4<br>±3.6 | | | | | 38 | 56.6<br>±7.0 | 60.3<br>±7.4 | 1.200 | 1.143 | 0.910 | 0.875 | 60 | **<br>95.5<br>± 9.9 | **<br>112.3<br>± 7.6 | 1.270 | 1.268 | 1.300 | 1.015 |
| 2 | 19 | **<br>27.5<br>± 5.2 | **<br>28.4<br>± 4.9 | 0.813 | 0.673 | 0.615 | 0.670 | 45 | 46.7<br>± 11.1 | 50.4<br>± 9.3 | 1.025 | 0.910 | 0.865 | 0.853 | 70 | 60.1<br>± 13.2 | **<br>73.9<br>± 10.2 | 1.175 | 1.185 | 0.927 | 0.845 |
| 3 | 19 | **<br>31.2<br>± 1.4 | 29.8<br>± 2.3 | 0.938 | 0.855 | 0.700 | 0.780 | 40 | 34.5<br>± 1.4 | 32.0<br>± 1.1 | 0.940 | 0.850 | 0.700 | 0.883 | 80 | **<br>84.6<br>± 4.2 | **<br>86.3<br>± 20.6 | 1.300 | | | 1.065 |
| 4 | | | | | | | | 45 | 56.7<br>± 9.4 | 60.5<br>± 11.2 | | | | | 53 | **<br>82.9<br>± 12.5 | **<br>92.0<br>± 13.2 | | | | |
| 5 | 19 | **<br>18.9<br>± 1.9 | **<br>17.6<br>± 1.6 | 1.145 | 0.192 | 0.785 | 0.880 | 40 | 37.5<br>± 5.0 | 41.1<br>± 4.8 | 1.067 | 0.997 | 0.900 | 0.838 | 73 | **<br>65.9<br>± 4.7 | **<br>70.7<br>± 4.8 | 1.225 | 1.080 | 0.930 | 0.897 |
| 6 | | | | | | | | 38 | 43.6<br>± 5.9 | 48.7<br>± 8.1 | 0.960 | 0.868 | 0.818 | 0.890 | | | | | | | |
| 7 | 21 | *<br>28.5<br>± 3.1 | *<br>29.8<br>± 3.8 | 1.005 | 0.830 | 0.855 | 0.880 | 40 | 31.4<br>± 5.8 | 32.4<br>± 6.2 | 1.030 | 0.900 | 0.913 | 0.915 | 71 | **<br>91.7<br>± 11.8 | **<br>98.9<br>± 12.0 | 1.205 | 1.273 | | 1.140 |
| 8 | 20 | 22.1<br>± 0.8 | *<br>21.7<br>± 1.6 | 0.935 | 0.843 | 0.722 | 0.785 | 40 | 27.3<br>± 6.8 | 33.4<br>± 10.0 | 1.005 | 0.923 | 0.748 | 0.735 | 80 | **<br>56.3<br>± 10.8 | **<br>66.7<br>± 8.9 | 1.030 | 1.105 | 1.085 | 0.978 |
| 9 | | | | | | | | 38 | 46.9<br>± 7.3 | 46.0<br>± 7.0 | 1.040 | 1.088 | 0.917 | 1.040 | 70 | **<br>83.2<br>± 10.3 | **<br>86.6<br>± 7.6 | 1.080 | 1.048 | 1.056 | 1.025 |

IDICA - intradural internal carotid, PPA - proximal pericallosal, DPA - distal pericallosal, MCA - Middle cerebral artery

t-Test Analysis: H/A and ISI hypocapnia and hypercapnia values were compared to the corresponding normocapnia values by a one-tailed t-test. A single asterisk denotes $p < .05$ while a double asterisk indicates $p < .01$. For example, for monkey #1, the 14.5 hypocapnia value and the 95.5 hypercapnia value were compared to the 56.6 normocapnia value and were both found significantly different at the 1 percent level.

Figure 23: Lateral angiograms of a control monkey during A. normocapnia; B. hypocapnia and C. hypercapnia. The sites at which vessel diameter measurements were made are indicated in A. Percent changes in rCBF (white values) in the various cerebral regions (detectors 1, 2, 3 and 5) are indicated. Black values indicate percent changes in vessel diameters.

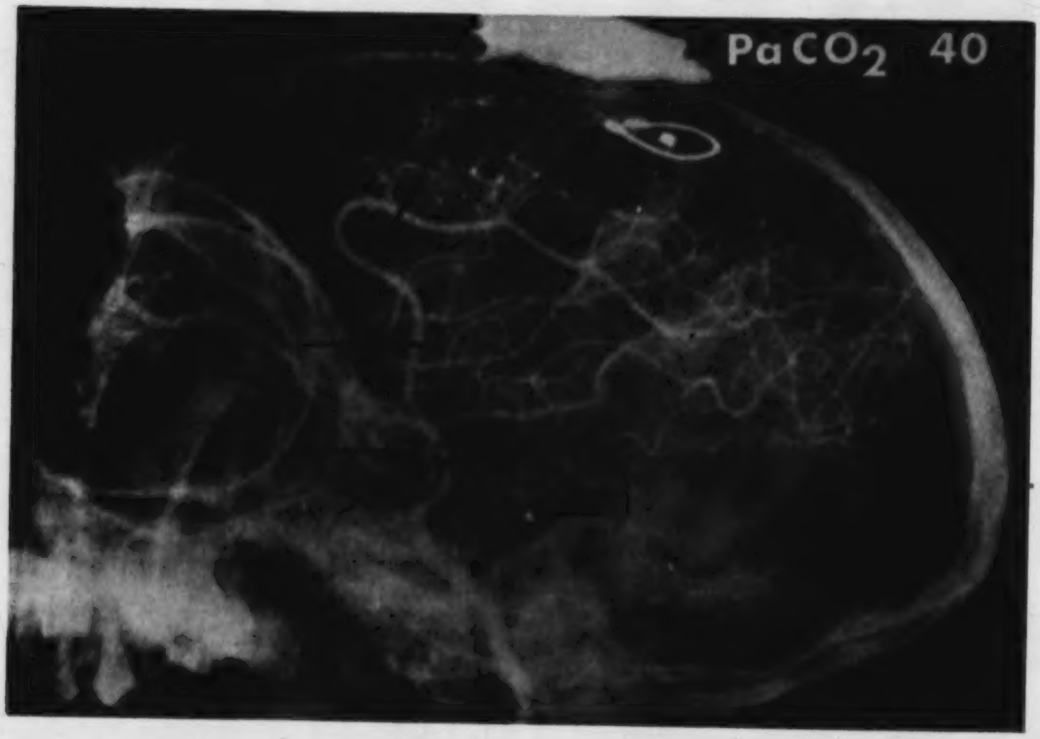


Figure 23A:

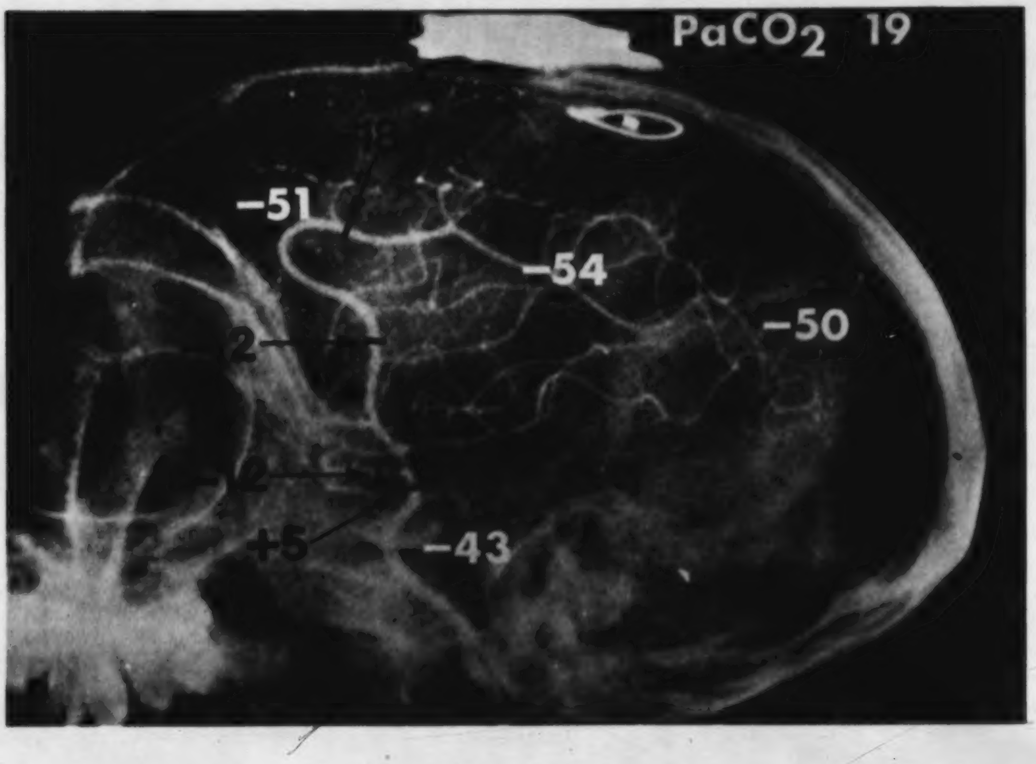


Figure 23B:

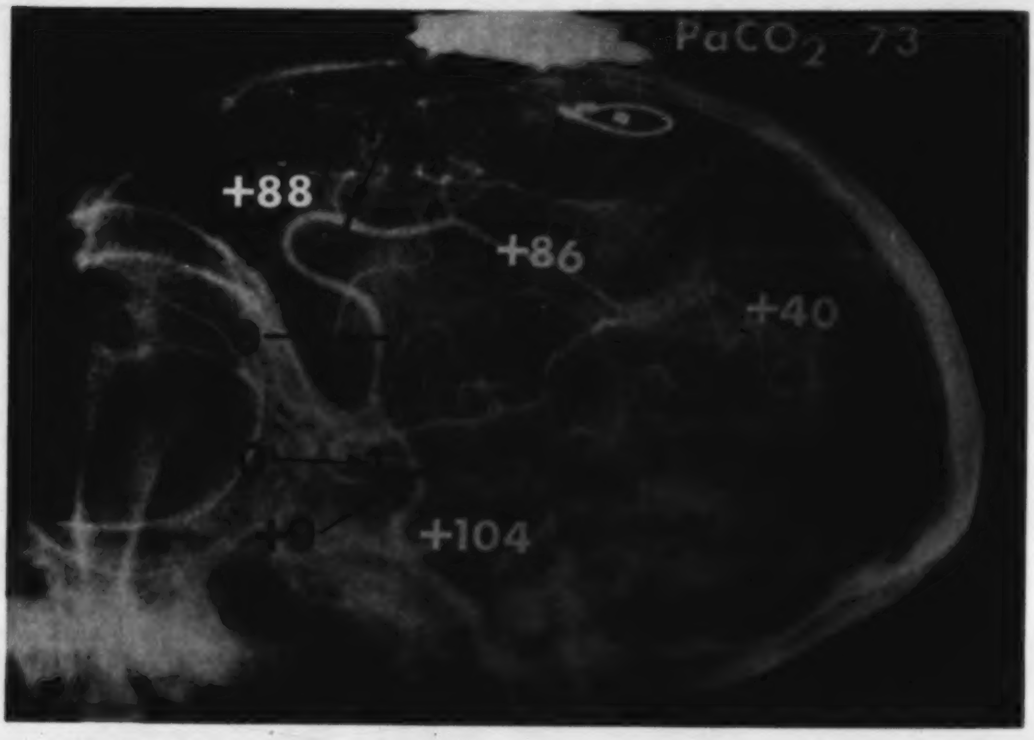


Figure 23C:

seven animals during induced hypercapnia. As shown in Table VII, all CBF values were significantly increased above normocapnic values, whereas consistent increases in vessel diameters were only observed in the more distal vessels (i.e., DPA), suggesting greater responsiveness of smaller vessels to $PaCO_2$ change.

### (d) Neurological Assessment

None of the control animals displayed neurological deficit at the termination of the experiments and were classified as Grade I or II. These animals were utilized for subsequent studies.

## B. Experimental Series

### (a) Subarachnoid Hemorrhage

In four animals, the effects of hypocapnia, normocapnia and hypercapnia on physiologic parameters before and after subarachnoid hemorrhage, were studied (Table VIII). Mean pre-subarachnoid hemorrhage $PaCO_2$ values during normocapnia and hypercapnia were 39.3 ± 1.4 mm Hg and 65.0 ± 5.1 mm Hg respectively. In the post-SAH period, corresponding $PaCO_2$ values were 40.3 ± 3.1 mm Hg and 65.2 ± 12.9 mm Hg. Post-subarachnoid hemorrhage $PaCO_2$ values during hypocapnia averaged 30.0 ± 2.0 mm Hg. Heart rate and mean blood pressure varied with $PaCO_2$ change in individual animals but not at significant levels ($p>0.05$). Changes in EKG produced by SAH included sinus arrhythmias nodal beats, nodal rhythm and premature ventricular contractions. These cardiac abnormalities were temporary and seldom lasted more than three minutes.

#### (i) Cerebral Blood Flow Response To SAH

Four animals were subjected to SAH during normocapnia by

## TABLE VIII.

## PHYSIOLOGIC RESPONSES TO HYPOCAPNIA AND HYPERCAPNIA IN THE EXPERIMENTAL SERIES.

| Group | Hypocapnia | | Normocapnia | | Hypercapnia | |
|---|---|---|---|---|---|---|
| SAH | Pre-SAH | Post SAH (n = 2) | Pre-SAH (n = 12) | Post-SAH (n = 12) | Pre-SAH (n = 6) | Post-SAH (= 10) |
| PaCO2 | | 30.00 ± 2.00 | 39.30 ± 1.40 | 40.3 ± 3.1 | 65.00 ± 5.10 | 65.20 ± 12.90 |
| pH | | 7.48 ± 0.04 | 7.39 ± 0.04 | 7.35 ± 0.06 | 7.22 ± 0.06 | 7.18 ± 0.03 |
| PaO2 | | 135 ± 7 | 120 ± 11 | 126 ± 14 | 118 ± 23 | 163 ± 26 |
| H.R. | | 107 ± 3 | 168 ± 36 | 145 ± 36 | 197 ± 22 | 142 ± 40 |
| M.B.P. | | 110 ± 5 | 121 ± 19 | 124 ± 17 | 132 ± 2 | 110 ± 18 |
| T.S. (ICA) | (n = 4) | | (n = 18) | | (n = 16) | |
| PaCO2 | 23.00 ± 6.60 | | 39.50 ± 2.10 | | 63.30 ± 11.50 | |
| pH | 7.49 ± 0.16 | | 7.36 ± 0.06 | | 7.15 ± 0.08 | |
| PaO2 | 142 ± 32 | | 129 ± 27 | | 125 ± 38 | |
| H.R. | 165 ± 30 | | 175 ± 45 | | 193 ± 51 | |
| M.B.P. | 100 ± 10 | | 111 ± 14 | | 124 ± 12 | |

SAH - Subarachnoid Hemorrhage; T.S.(ICA) - Traumatic Spasm of Internal Carotid Artery injection of autogenous blood into the chiasmatic cistern. Hemorrhage produced a significant decrease in CBF in three animals and a non-significant reduction in the fourth (Table IX). These CBF changes were global in nature with no one area being affected to a greater degree. As shown in the previous studies, little subsequent variation in rCBF was observed in the post-SAH period during normocapnia. The average reduction in CBF varied from 15 percent (monkey 11) to 25 percent (monkey).

### (ii) Post-Subarachnoid Hemorrhage CBF Response to $PaCO_2$ Change

In two animals, hypocapnia induced in the post-SAH period further decreased CBF, suggesting that vessel reactivity to $PaCO_2$ decrease was present after SAH.

Graded hypercapnia in the post-SAH period resulted in a gradual increase in CBF. As shown by regression analysis (Figure 24), the response to $PaCO_2$ change was less marked when compared to control and pre-SAH studies. However when $PaCO_2$ values were increased to about 60-65 mm Hg, a much greater increase in CBF occurred with values approaching or surpassing pre-SAH normocapnia flow rates (Figure 25). This "CBF breakthrough" occurred in all monkeys subjected to SAH.

### (iii) Vessel Caliber Responses

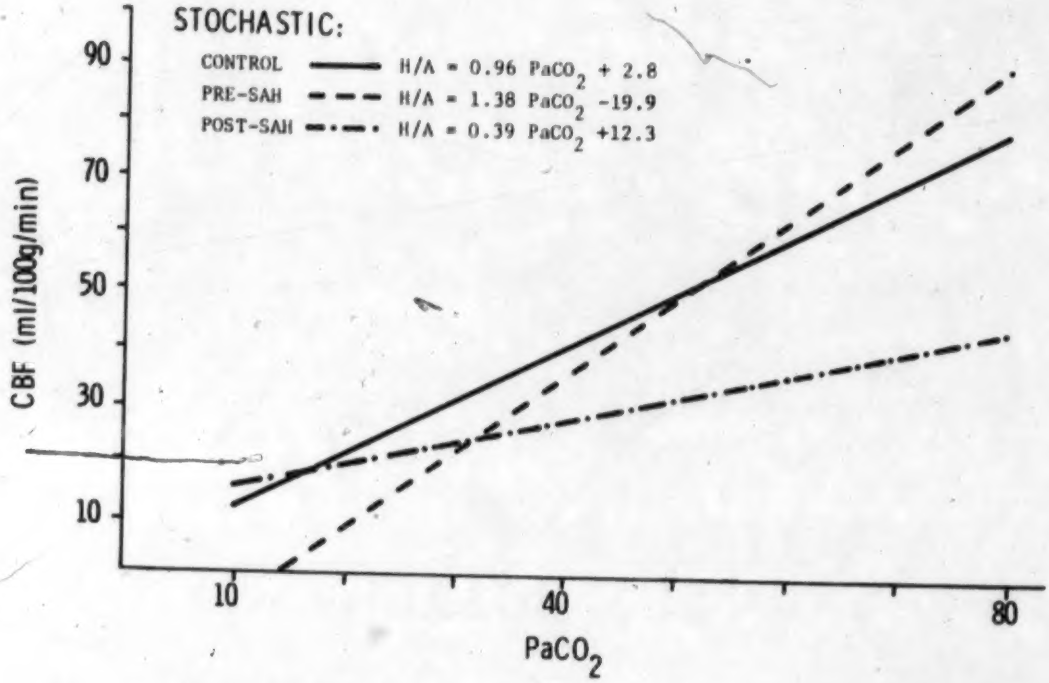
Serial angiographic studies were performed (during normocapnia) at the onset of the experiments and after insertion of the needle into the chiasmatic cistern. No significant variation ($p > 0.05$) in intradural vessel diameter was produced by needle insertion. Post-SAH angiograms were obtained immediately after completion of the first CBF study and at the extreme $PaCO_2$ values. Additional angiographic studies

## TABLE IX.

## MEAN HEMISPHERIC BLOOD FLOW (H/A AND ISI ANALYSIS) AND VESSEL CALIBER (mm) RESPONSES TO HYPOCAPNIA AND HYPERCAPNIA IN INDIVIDUAL ANIMALS SUBJECTED TO SAH

| | | Hypocapnia | | | | | | | Normocapnia | | | | | | | Hypercapnia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M# | | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA |
| 10 | Pre SAH | | | | | | | | 40 | 25.4<br>± 6.0 | 29.9<br>± 7.3 | 0.983 | 1.158 | 0.918 | 0.878 | 60 | * 44.1<br>± 11.1 | * 53.2<br>± 15.8 | | | | |
| | Post SAH | | | | | | | | 42 | 20.7<br>± 1.3 | 22.2<br>± 1.9 | 0.830 | 0.922 | 0.705 | 0.738 | 66 | * 36.1<br>± 3.5 | 41.8<br>± 6.6 | 0.800 | 0.847 | 0.743 | 0.603 |
| 11 | Pre SAH | | | | | | | | 40 | 28.1<br>± 2.3 | 25.2<br>± 2.1 | 1.053 | 0.930 | 0.803 | 0.803 | | | | | | | |
| | Post SAH | 32 | ** 17.9<br>± 1.4 | ** 18.1<br>± 1.7 | | | | | 35 | * 20.8<br>± 2.6 | 20.9<br>± 3.1 | 0.768 | 0.785 | 0.888 | 0.773 | 60 | 33.3<br>± 9.1 | * 37.2<br>± 7.1 | 1.155 | 0.883 | 0.838 | 0.860 |
| | | | | | | | | | 39 | ** 19.7<br>± 2.0 | * 20.8<br>± 2.0 | 1.085 | 0.885 | 0.828 | 0.820 | 83 | ** 90.2<br>± 6.2 | ** 50.0<br>± 5.0 | 1.108 | 1.083 | 1.210 | 0.963 |
| 12 | Pre SAH | | | | | | | | 39 | 40.2<br>± 5.6 | 42.5<br>± 6.9 | 1.056 | 0.910 | 0.913 | 1.025 | 62 | ** 82.2<br>± 20.0 | * 83.8<br>± 23.9 | | | | |
| | Post SAH | 21 | ** 26.5<br>± 1.5 | ** 26.4<br>± 2.5 | 1.003 | 0.913 | 0.783 | 0.886 | 40 | * 31.4<br>± 2.6 | * 27.5<br>± 6.6 | 1.018 | 0.912 | 0.828 | 0.938 | 81 | * 74.0<br>± 20.2 | * 86.7<br>± 34.7 | 1.163 | 1.083 | 1.082 | 1.110 |
| 13 | Pre SAH | | | | | | | | 39 | 46.9<br>± 7.3 | 46.0<br>± 7.00 | 1.040 | 1.088 | 0.917 | 1.040 | 70 | ** 83.2<br>± 10.2 | ** 88.6<br>± 7.1 | 1.080 | 1.048 | 1.055 | 1.025 |
| | | | | | | | | | | | | | | | | 81 | ** 83.5<br>± 11.6 | ** 87.6<br>± 6.3 | | | | |
| | Post SAH | | | | | | | | 40 | ** 29.6<br>± 3.3 | ** 30.7<br>± 4.8 | 0.887 | 0.783 | 0.753 | 0.800 | 65 | 39.5<br>± 1.4 | * 35.5<br>± 2.4 | 0.880 | 0.863 | 0.730 | 0.733 |
| | | | | | | | | | | | | | | | | 81 | 61.5<br>± 9.9 | 70.3<br>± 7.1 | 0.935 | 0.962 | 0.818 | |

H/A and ISI hypocapnia (post-SAH) and hypercapnia (pre- and post-SAH) values are compared to the corresponding normocapnia (pre- and post-SAH) values by a two-tailed t-test. A single asterisk denotes $p < .05$, while a double asterisk indicates $p < .01$.



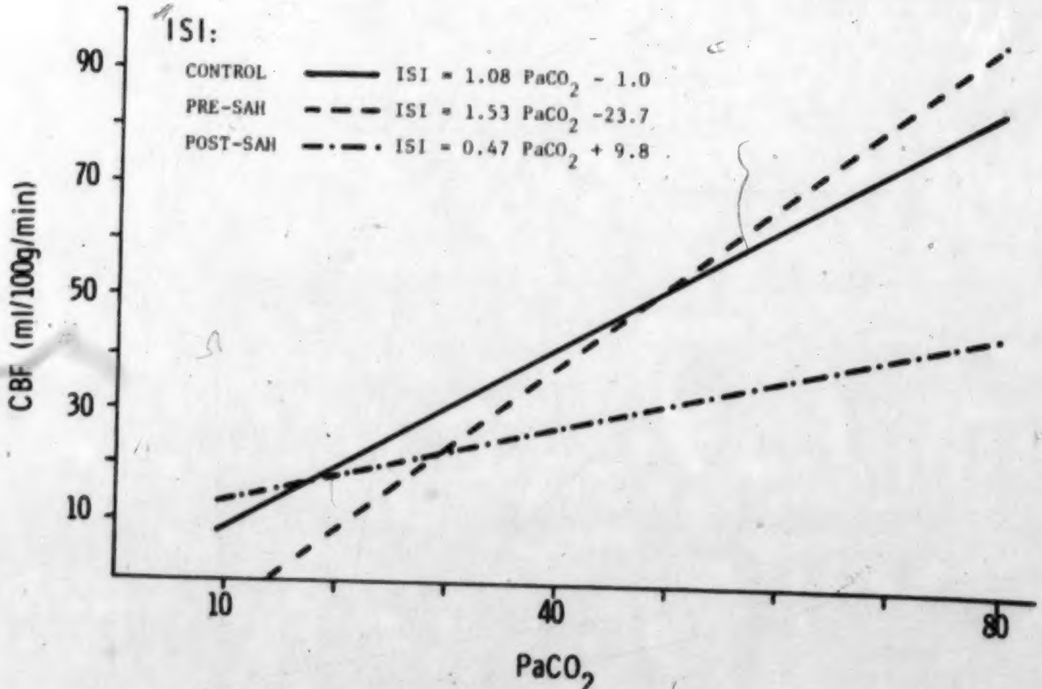


Figure 24: Regression line analysis for stochastic (H/A) and initial-slope-index (ISI) flows in the PaCO2 range 10-80mm Hg for control, pre-SAH and post-SAH monkeys.

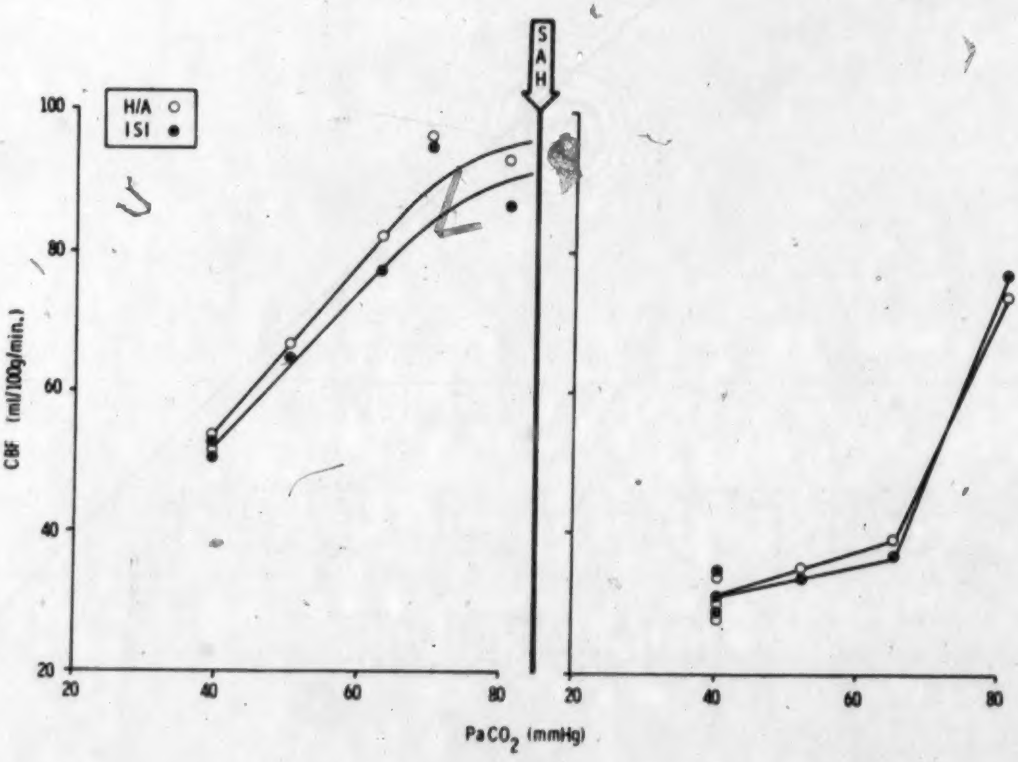


Figure 25: CBF (H/A and ISI) responses to graded hypercapnia in the pre-SAH and post-SAH period. Note the marked increase in post-SAH CBF when PaCO2 is increased above 65mm Hg (breakthrough phenomenon).

were carried out whenever marked changes in CBF occurred during induced increases in $PaCO_2$.

Subarachnoid hemorrhage produced a decrease in intradural vessel caliber in all animals. The degree of vasospasm differed among different animals and also in different vessels in the same animal (Table IX). In all animals, marked reflux into the opposite ICA and vertebral arteries occurred, suggesting increased cerebrovascular resistance.

Vessel reactivity was tested in one animal subjected to hypocapnia in the Post-SAH period. Further vessel constriction occurred suggesting that maximal decrease in vessel diameter was not produced by SAH.

With hypercapnia, post-subarachnoid hemorrhage CBF was invariably increased and reflux abated, although vessel diameter responses were highly variable. In one animal (monkey 10), hypercapnia ($PaCO_2$ 66 mm Hg) produced a paradoxical response and increased vasospasm over the post-SAH normocapnic level. Even though hypercapnia intensified the vasospasm, CBF increased above the pre-SAH normocapnic flow (Figure 26). In another animal (monkey 13), hypercapnia caused a mild relaxation of post-SAH vasospasm, but the vessel diameters were still below pre-SAH normocapnia diameters. Although vasospasm persisted during severe hypercapnia, reflux of contrast material disappeared and CBF values increased above pre-SAH normocapnia flow rates (Figure 27).

In two monkeys, graded hypercapnia progressively increased the distal intradural vessel diameters. At high $PaCO_2$ levels. (60-70 mm Hg) vasospasm disappeared and at extreme $PaCO_2$ values (near 80 mm Hg),

Figure 26: Pre- and post-SAH lateral angiograms of monkey 10.

A. Pre-SAH normocapnic angiogram;
B. Post-SAH normocapnic angiogram displaying vasospasm;
C. Post-SAH angiogram during hypercapnia (PaCO2 66mmHg) showing intensified vasospasm even though rCBF increased. White values - rCBF; black values - vessel diameters.

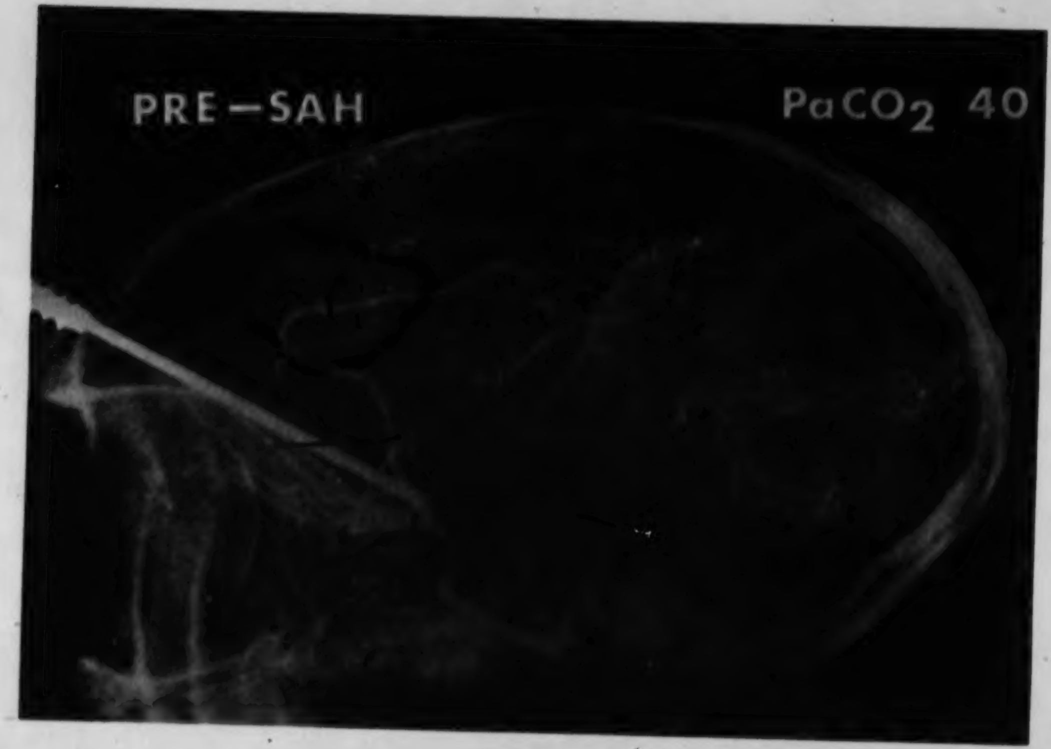


Figure 26A:

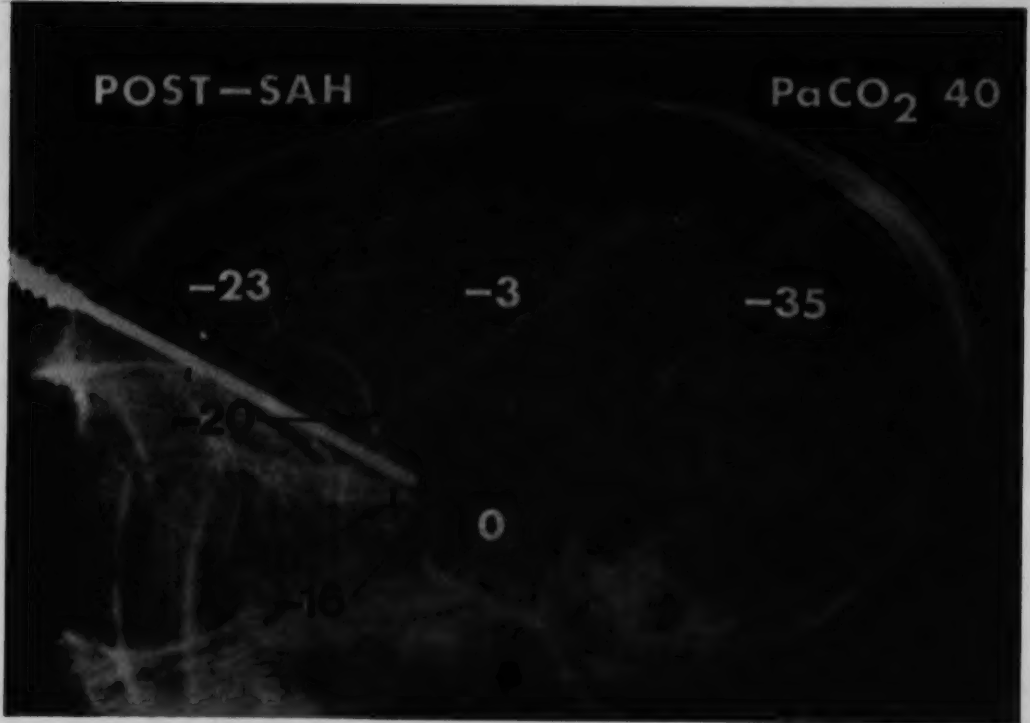


Figure 26B:

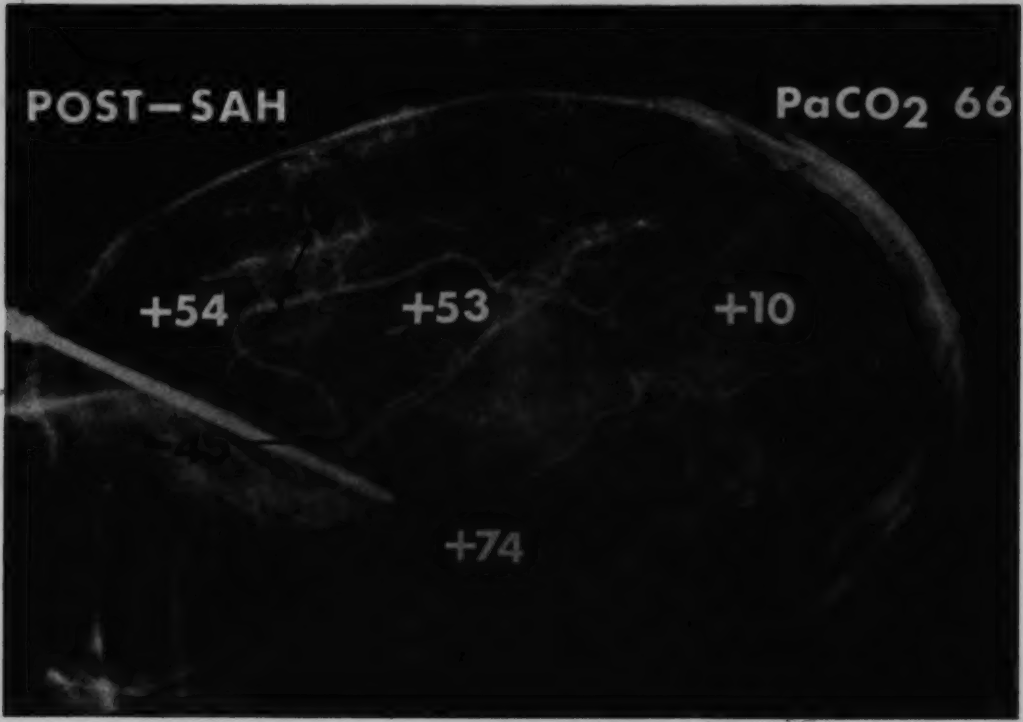


Figure 26C:

Figure 27: Pre- and post-SAH angiograms of monkey 13. White values represent rCBF; black values show percent change in vessel diameters.

A. Pre-SAH angiogram during normocapnia;

B. Pre-SAH angiogram during hypercapnia showing marked increase in rCBF whereas vessel diameters are little affected:

C. Post.SAH angiogram showing vasospasm and decreased rCBF;

D. Post-SAH angiogram during hypercapnia showing increased rCBF values even though vessel diameters remain below control dimensions.

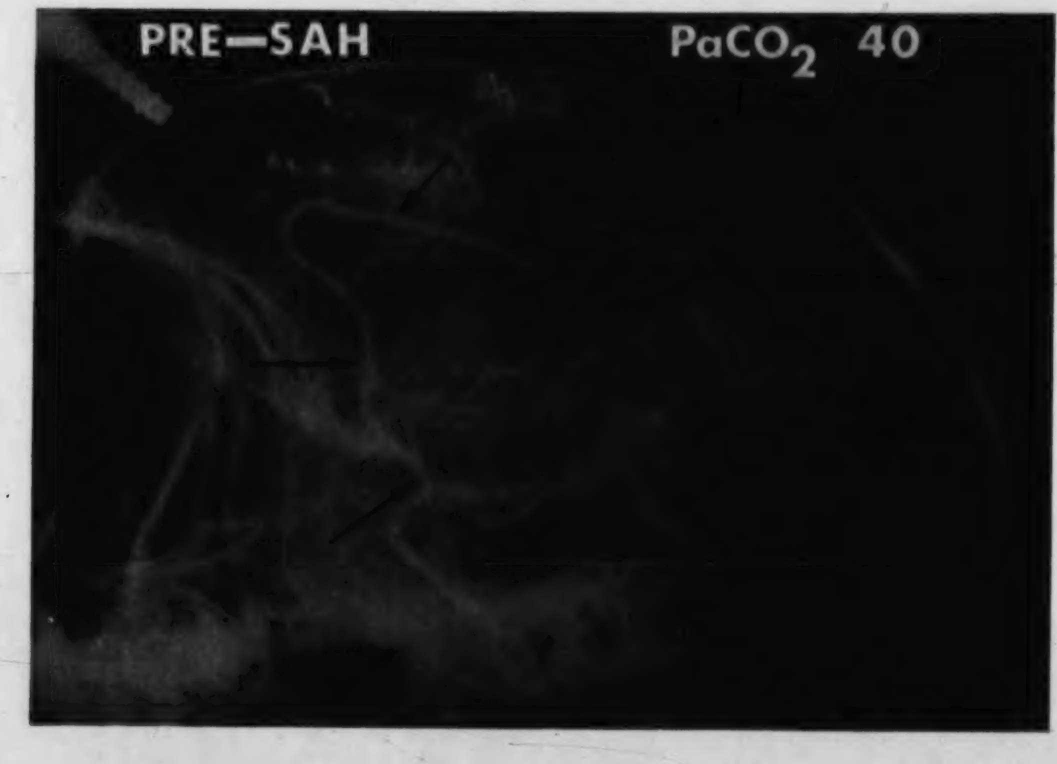


Figure 27A:

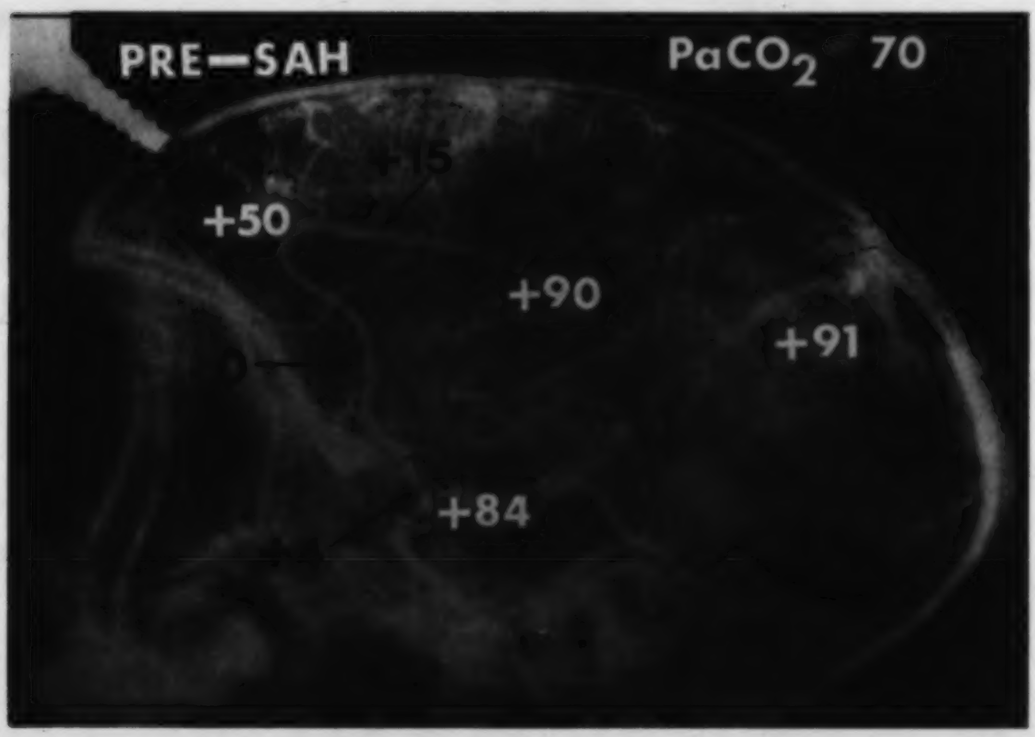


Figure 27B:

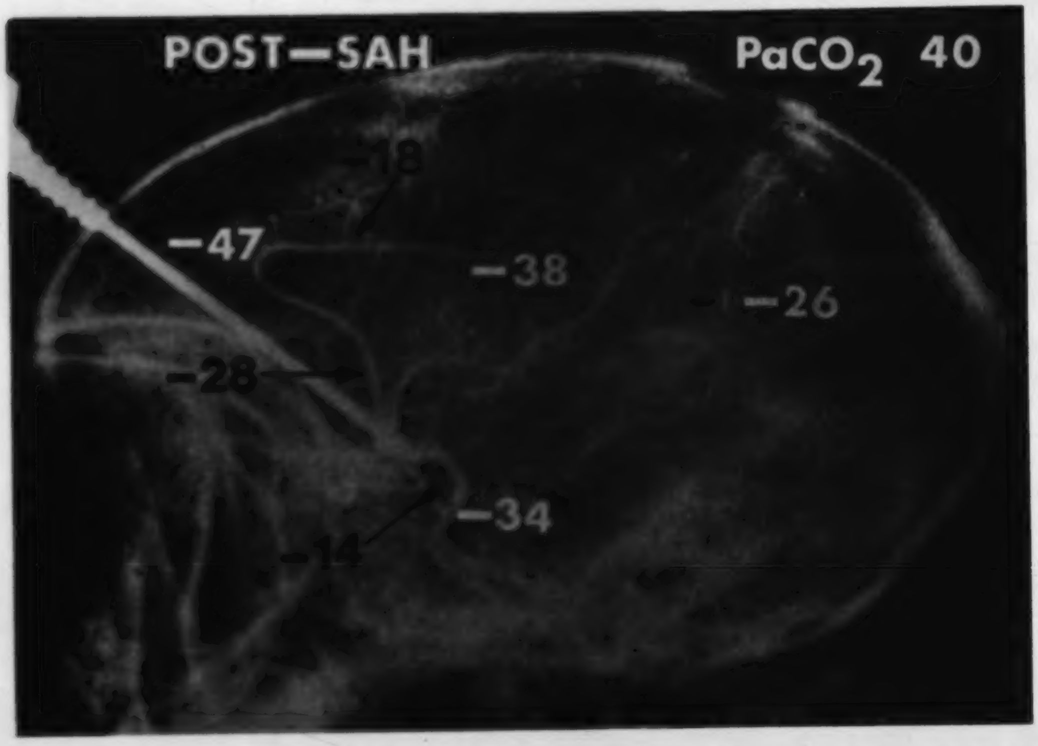


Figure 27C:

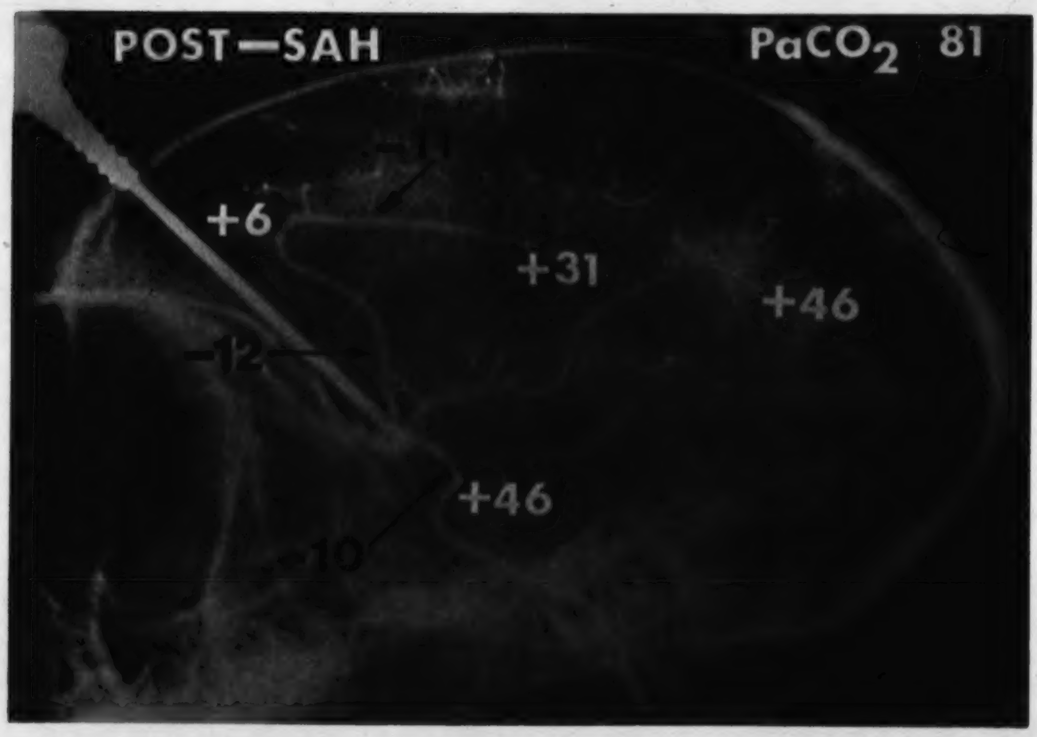


Figure 27D:

vessel calibers increased to above the pre-SAH normocapnic diameters. However, an increase in vessel diameter above pre-SAH control values was demonstrated only in the distal pericallosal arteries, ~~again~~ suggesting the increased sensitivity of the small distal vessels to $PaCO_2$ change.

#### (iv) Neurological Assessment

The animals were assessed neurologically and three were classed as Grade III and one, Grade II. The animals were sacrificed and the brains removed for verification of subarachnoid hemorrhage (Figure 28).

### (b) Traumatic Spasm of the Internal Carotid Artery

In four animals, the effect of $PaCO_2$ change on pH, $PaO_2$, HR and MBP was measured and the results are given in Table X . Average $PaCO_2$ values during hypocapnia, normocapnia and hypercapnia were 23.0 ± 6.6 mm Hg, 39.5 ± 2.1 mm Hg and 63.3 ± 11.5 mm Hg respectively. Mean HR and MBP values were reduced in hypocapnia and increased during hypercapnia but not to levels of statistical significance ($p>0.05$).

#### (i) Relationship Between CBF and $PaCO_2$

Mean hemispheric blood flow (H/A and ISI) values in four monkeys displaying traumatic spasm of the internal carotid artery average 29 ± 4 ml/100gm/min. Response to induced hypocapnia was tested in three animals (Table X ) and a decrease in CBF was observed in two (significant decrease present in one animal). In the fourth animal (monkey 16), a minute amount of air was injected into the internal carotid artery at the onset of the experiment and in this animal, induced hypocapnia increased CBF from the normocapnia values (paradoxical response).

Figure 28: Photographs of monkey brain showing diffuse SAH: (A) Basal view; (B) Posterior view.

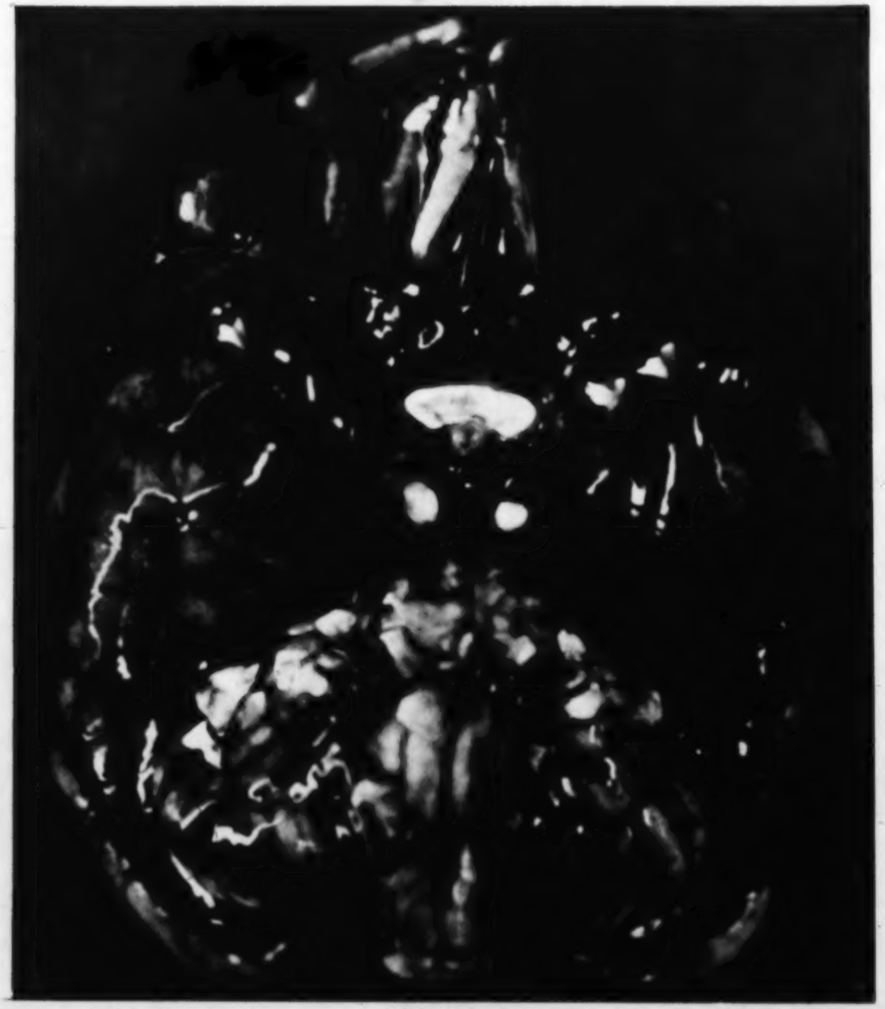


Figure 28A.

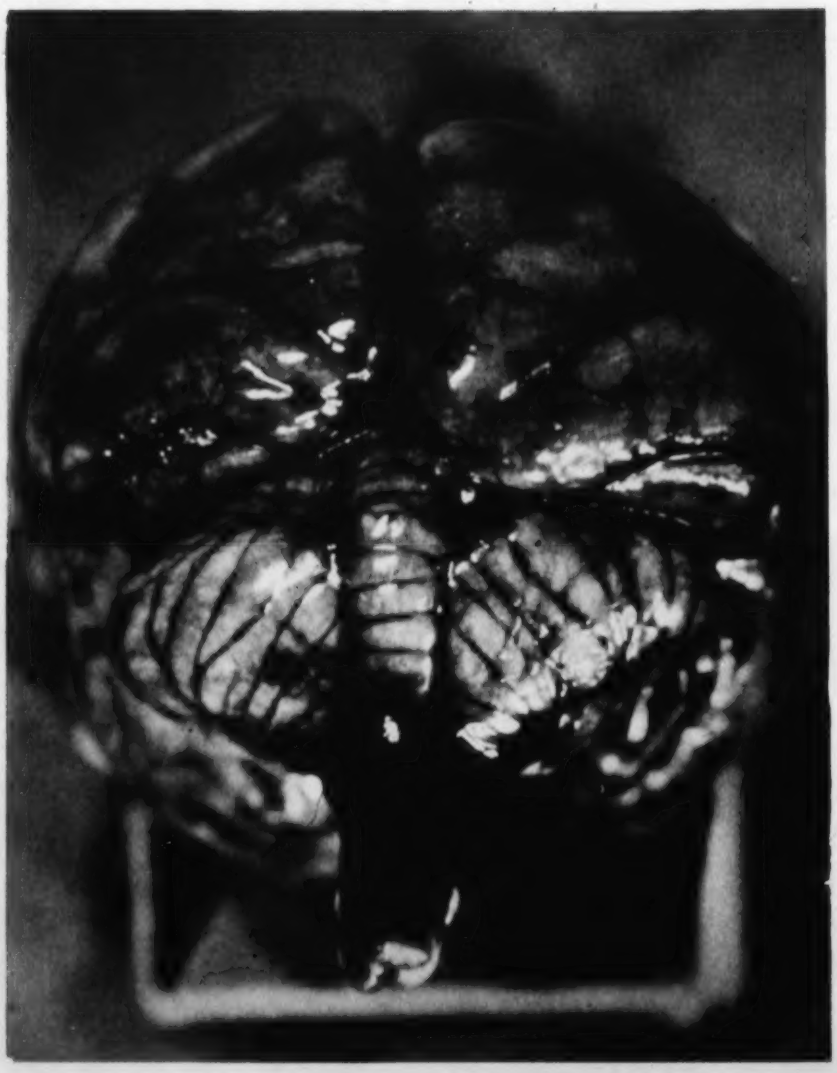


Figure 28B:

## TABLE X.

### MEAN HEMISPHERIC BLOOD FLOW (H/A AND ISI ANALYSIS) AND VESSEL RESPONSES TO HYPOCAPNIA AND HYPERCAPNIA IN INDIVIDUAL ANIMALS SUBJECTED TO TRAUMATIC INTERNAL CAROTID ARTERY SPASM

| | Hypocapnia | | | | | | | Normocapnia | | | | | | | Hypercapnia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M # | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA | PaCO2 | H/A | ISI | IDICA | PPA | DPA | MCA |
| 14 | 19 | * 24.5<br>±4.3 | ** 22.3<br>± 5.1 | 1.025 | 0.950 | 0.822 | 0.723 | 40 | 32.4<br>± 3.1 | 33.7<br>±3.2 | 1.190 | 1.050 | 0.895 | 1.006 | 71 | ** 52.5<br>± 2.5 | ** 62.8<br>± 3.5 | 1.270 | 1.110 | 1.230 | 1.140 |
| 15 | 20 | 29.0<br>± 8.8 | 35.2<br>13.0 | 0.875 | 0.810 | 0.713 | 0.773 | 40 | 34.4<br>± 2.7 | 37.1<br>± 1.9 | 1.025 | 0.850 | 0.720 | 0.715 | 70 | * 64.1<br>± 14.4 | ** 73.6<br>± 8.7 | 0.843 | 0.935 | 0.732 | 0.823 |
| | | | | | | | | | | | | | | | 79 | * 60.9<br>± 13.4 | ** 75.7<br>± 9.0 | 0.975 | 1.135 | 0.863 | 1.028 |
| 16 | 16 | 32.5<br>± 8.8 | 32.4<br>± 7.9 | | | | | 40 | 19.4<br>± 5.3 | 25.3<br>± 6.8 | | | | | 70 | 20.2<br>± 9.3 | 27.9<br>± 11.6 | | | | |
| 17 | | | | | | | | 36 | 22.7<br>± 5.5 | 29.6<br>±7.4 | 0.985 | 0.876 | 0.710 | 0.900 | 63 | 30.7<br>± 1.4 | 42.2<br>±5.9 | 1.305 | 1.235 | 1.078 | 1.373 |
| | | | | | | | | | | | | | | | 80 | 25.5<br>± 1.3 | 38.0<br>± 2.2 | 1.295 | 1.138 | | 1.230 |
| | | | | | | | | | | | | | | | 85 | * 38.2<br>± 3.7 | **51.9<br>± 3.2 | 1.388 | 1.170 | 1.048 | 1.353 |

t-Test Analysis: H/A and ISI hypocapnia and hypercapnia v:lues were compared to the corresponding normocapnia values by a two-tailed t-test.
A single asterisk denotes p < .05 while a double asterisk indiectes p < .01.

In four animals, CBF studies were performed during hypercapnia. In three, a significant increase in CBF occurred, although in one animal marked elevation was not exhibited until a $PaCO_2$ value of 85 mm Hg was reached.

### (ii) Vessel Diameter Responses to $PaCO_2$ Change

Angiographic studies were obtained in two animals during hypocapnia and generalized vessel constriction was demonstrated in both cases. Severe vasoconstriction was associated with marked CBF reduction and mild constriction with a smaller CBF decrease.

Vessel diameter measurements were made in three animals during hypercapnia. Increase in $PaCO_2$ generally caused vasodilation, but the degree of reponses was variable. In two animals (monkey 14 and 15, Table 10), only the MCA increased in diameter, yet a global increase in CBF occurred. In monkey 17, hypercapnia ($PaCO_2 > 60$ mm Hg) resulted in generalized marked vasodilation of the larger intradural vessels, although the increase in CBF was not significant until $PaCO_2$ values of 85 mm Hg were reached. These correlative studies suggest that the caliber of the large intradural vessels, as demonstrated by angiography, do not adequately reflect the status of cerebral tissue perfusion.

### (iii) Neurological Assessment

Two animals were classified as Grade IV, one as Grade III and one, Grade II. A good correlation between resting CBF (during normocapnia) and neurological grade was present. The Grade IV animals had the lowest and the Grade II animal had the highest resting CBF values.

# CHAPTER FIVE

## DISCUSSION

Since its first documentation in 1949 by Robertson (140), the problem of cerebral vasospasm has occupied a central position in the management of ruptured intracranial aneurysms. Robertson concluded that ischemic lesions in patients with subarachnoid hemorrhage occasionally were caused by arterial spasm and that "this mechanism is far commoner than realized." In 1951, Ecker and Riemenschneider (104) demonstrated with angiographical methods vasospasm of the major cerebral vessels in patients with aneurysms of the circle of Willis. With refined angiographical techniques, the incidence of cerebral vasospasm associated with ruptured intracranial aneurysms has been reported to be between 30 and 68 percent (141, 142, 143). Pool (1958) postulated that the clinical course following rupture of an aneurysm was dependent to a large degree on the presence, extent and duration of cerebral vasospasm (98).

Numerous recent retrospective clinical studies have indicated a direct causal relationship between cerebral vasospasm, frequency of infarction and increased morbidity and mortality in patients suffering from SAH (141, 144, 145, 146). Vasospasm, when demonstrated, has been asserted to be a contraindication to definitive surgical intervention (146, 147). Although the literature on arterial vasospasm after subarachnoid hemorrhage has increased immensely, the fundamental etiology of vasospasm remains to be fully elucidated.

With the introduction of physiological methods of quantitatively determining cerebral blood flow (inert gas washout technique of Ingvar and Lassen), renewed interest in the relationship between cerebral vasospasm, perfusion and function was stimulated. Kagstrom (148) demonstrated a 20 percent reduction in hemispheric blood flow in seven patients examined during the first few weeks after SAH. A three-month follow-up study revealed a correlation between hemispheric flows and the severity of neurological sequelae. James(25) found reduced cerebral perfusion in 36 patients suffering from recent subarachnoid hemorrhage. The reduction in CBF correlated with the impairment of consciousness and the radiological appearance of spasm of large cerebral arteries. However, Zingesser, et al.(149) were unable to obtain a correlation between rCBF and angiographical vasospasm in 19 patients with SAH. They found a reduction in cerebral perfusion even in the absence of arterial constriction. Furthermore, vasospasm when present, was not necessarliy associated with reduced cerebral perfusion in the vascular territory distal to the spasm. Ferguson, et al. (150) studied 22 patients with SAH and found an excellent correlation between the neurological state of patients and cerebral blood flow. However, in comparing patients with vasospasm (8 cases) to those without spasm, no signficant difference in cerebral perfusion was detected. They concluded that the significance of arterial vasospasm was debatable, although severe spasm was usually associated with a marked decrease in CBF. Symon, et al. (151) found a correlation between cerebral blood flow and the clinical condition of patients. Although they concluded that a good correlation between angiographical abnormalities (spasm, partial occlusion as a complication of surgery, areas of apparent slowed flow) and rCBF was present, only in two of four cases was there a correlation between vasospasm and cerebral perfusion.

Heilbrun (152) performed pre-operative and post-operative rCBF studies in 14 patients suffering from SAH. All patients demonstrated a global reduction in cerebral perfusion. Of 5 patients studied in the pre-operative period, 3 demonstrated focal ischemia without evidence of spasm. Seven patients were studied in the post-operative period and 4 demonstrated spasm and reduced rCBF in the distribution of the spastic arteries. However, in comparing patients with severe and mild spasm, no difference in rCBF was found. These authors concluded that because there was a global reduction in cerebral perfusion after SAH, this reduced CBF could not be attributable directly to arterial spasm since spasm was absent in 5 of 6 pre-operative and 4 of 8 post-operative studies. In addition, vasospasm when present was always localized.

Hashi, et al. (153) concurrently studied CBF and angiographical changes in baboons after induced SAH. Seven of ten baboons displayed angiographical vasospasm but there was no significant difference in CBF in animals with or without arterial spasm in the acute phase after SAH. At 24-48 hours, post-SAH spasm was still present but a significant reduction in cerebral perfusion occurred. These investigators concluded that reduced CBF was the result of perivascular edema and vasospasm. Yamaguchi and Waltz (154) studied CBF in cats after puncture of the middle cerebral artery and found no constant relationship between CBF and the calibers of surface cortical arteries.

From these studies it is evident that the pathophysiological mechanisms involved in the production of decreased cerebral perfusion following subarachnoid hemorrhage remain to be fully elucidated. While marked cerebral vasospasm appears to be frequently associated with diminished cerebral blood flow, other factors (metabolic and toxic) may be of importance.

The clinical studies have stimulated research on subarachnoid hemorrhage and interest continues in developing improved models for studying arterial spasm and cerebral perfusion. Furthermore, such studies may be useful in evaluating the effectiveness of therapeutic agents designed to restore cerebral perfusion and the clnical grade of patients after subarachnoid hemorrhage.

The present experimental studies were designed to critically investigate the relationship between induced subarachnoid hemorrhage and its acute effect on cerebral perfusion, intradural arterial caliber and the neurological condition of the animals.

Rhesus monkeys (macaca mulatta) were chosen for investigation because of the striking anatomical similarity between this animal's cerebral vasculature and that of man. The animals were sedated with pentobarbital, using doses which do not measurably affect cerebral blood flow and the cerebral metabolism (155). Anesthesia was maintained with nitrous oxide-oxygen and curare, a regimen previously shown not to affect cerebral perfusion nor to alter the responsiveness of cerebral perfusion to carbon dioxide change (156). Cerebral angiographical studies were performed in studies B and C by injection of Meglumine iothalamate into the internal carotid artery. Potchen, et al. (157) showed that a consistent but non-uniform change in CBF occurred after Conray 60 angiography and suggested that the effect disappeared in approximately 30 minutes (although the optimal waiting period was not determined). In the present studies, cerebral perfusion studies were almost invariably performed prior to angiography. On occasion when angiography preceded CBF studies during steady state conditions no significant change in cerebral perfusion ($p>0.05$) was detected after a waiting period of 15 minutes.

In the initial study (study A), an accurate reproducible and quantitative method of measuring cerebral blood flow in the rhesus monkey was developed. In general, an excellent correlation between the stochastic and initial - slope - index methods was obtained and these flow values were utilized for statistical analysis. Similar findings have been reported by Hoedt-Rasmussen (158) and Wilkinson (159). Cerebral perfusion values remained constant over the 5 to 6 hour experimental period in control animals. Prolonged immobilization did not affect CBF values as reported by Raichle, et al. (160). Furthermore, the intra-arterial residue detection technique was reproducible as demonstrated by repeat CBF studies in the same animals after an interval of one to two weeks.

Subarachnoid hemorrhage was simulated by the method described by Weir and his associates (161). With increased experience, this method, although not the exact replica of clinical aneurysmal rupture, was found to be simple, relatively atraumatic and reliable. Introduction of fresh autogeneous arterial blood into the chiasmatic cistern caused a significant reduction in cerebral perfusion in approximately 80 percent of animals. The reduction in CBF was acute and remained unaltered for the duration of the experimental period. A bi-phasic response as described by Brawley (112) and Simeone (162) was not observed because of the short duration of the experiments.

The pathogenesis of cerebral ischemia after SAH was not evident in the initial study. However, decreased cerebral perfusion did not appear to be caused by peripheral or central cardiovascular abnormalities as the changes observed were transient in nature. The pathogenesis of the cardiac abnormalities was not investigated although recent studies have shown that the hypothalamus is in some way affected in subarachnoid hemorrhage. This hypothalamic sympathetic stimulation apparently causes intramyocardial release of catecholamines and these substances appear to produce electrocardiographic changes and occasionally subendocardial ischemia (163).

Animals subjected to subarachnoid acidic saline injection demonstrated an increase in cerebral perfusion lasting one hour or more. None of the monkeys exhibited a decrease in CBF over the duration of the experiments. Although the series is small and subject to statistical criticism, this finding is consistent with other studies (47,48, 164) which have demonstrated increased cerebral perfusion following a reduction in the pH of the interstitial fluid (CSF) of the brain. Furthermore, the demonstration of no decrease in cerebral perfusion after rapid injection of saline into the subarachnoid space supports the concept that traction and mechanical factors are of little importance in initiation and propagation of cerebral vasaospasm.

The excellent correlation between CBF, electroencephalographic and neurological status of the animals supports the findings of recent clinical investigations.

The second study (study B) was designed to concurrently investigate changes in regional cerebral blood flow and arterial vessel caliber following induced SAH. The changes observed were subsequently compared with the clinical and neurological state of the animals following termination of each experiment. With this study, the relationship between cerebral vasospasm and change in cerebral perfusion was assessed by statistical methods.

Measurement of regional cerebral blood flow (rCBF) was achieved using a multi-detector scintillation counting system designed and adapted for studies in the rhesus monkey. In control animals, flow values recorded from the frontal,central, parietal and temporal areas were not significantly different from each other. However, flow within the cerebellum was decreased by approximately 25 percent while orbito-maxillary tissue flow was reduced by 35 to 50 percent. Similar results were reported by Hoedt-Rasmussen for man (158).

Induced SAH caused a significant global reduction of CBF in approximately 75 percent of animals. This reduction was immediate and flow values remained significantly reduced for the duration of the experiments. Little variation in rCBF occured after reduced perfusion was established. Significant generalized vasospasm of the larger intradural arteries was exhibited in all animals subjected to SAH. The degree of vasospasm present in animals not exhibiting a decrease in rCBF was not statistically different from those demonstrating a significant decrease in rCBF. Furthermore, this study demonstrated that cerebral blood flow is more constantly related to the clinical grade of the animals than the degree of arterial constriction after SAH. Since the clinical grade of patients suffering from aneurysmal rupture is a very important factor in determining the course and prognosis (165), this relationship appears important.

The apparent discrepancy between vasospasm and cerebral perfusion has not been fully resolved in the present experiments.

However, these studies suggest that vessels of subradiological caliber are more important in regulation and maintenance of cerebral perfusion than are the larger capacitance vessels. Measurement studies with statistical application have shown that the distal vessels are more vasoreactive to SAH. Similar findings were reported by Raper (166) and Symon (167). Moreover, indirect evidence arising from the present experiments suggests that other factors may play a role in the development of cerebral ischemia after SAH. Animals which displayed low flow values and marked neurological deficit also exhibited increased intracranial pressures in the post-SAH period. This finding is in accordance with the study of Hashi, et al. (153), These investigators suggested that cerebral ischemia after SAH may result from swelling of perivascular astrocytes and cerebral edema.

The presence of an excellent correlation between cerebral blood flow and the neurological state suggests that perfusion studies are more sensitive than the degree of vessel caliber constriction with respect to cerebral function and survival after subarachnoid hemorrhage. Further experimental and clinical studies are required to determine whether angiographical spasm in the presence of normal cerebral perfusion and a good clinical grade is still a contraindication to early aneurysmal surgery.

The final study (study C) was designed to concurrently investigate regional cerebral blood flow and intradural vessel responses to graded carbon dioxide tension change in control monkeys and in monkeys subjected to SAH and traumatic internal carotid artery spasm.

It is well accepted that carbon dioxide has the most profound effect on cerebrovascular tone of any substance yet investigated. Numerous clinical (168,169,170) and experimental (41,42, 171) studies have shown that increase in $PaCO_2$ causes an increase in CBF in normal brain tissue and a decrease in $PaCO_2$ causes a decrease in CBF. However, the effect of $CO_2$ on cerebral hemodynamic responses in ischemic brain tissue has provided inconsistent results and interpretations (171,172 173, 174).

In control animals, cerebral perfusion increased linearly between $PaCO_2$ values of 30 mm Hg and 60 mm Hg and within this range cerebrovascular reponsiveness was maximal. Induced hypocapnia to approximately 20 mm Hg resulted in a 40 percent derease in cerebral perfusion. Below $PaCO_2$ values of 30 mm Hg, CBF resonse was considerably attenuated, but below 25 mm Hg a small increase in CBF was seen . Increases in CBF during extreme hypocapnia have been shown to be due primarily to hypoxia created by intense cerebral vasoconstrction (46).

Increase in $PaCO_2$ from 40 mm Hg to 62 mm Hg resulted in 75 percent increase in CBF. These findings are similar to those obtained in man by Kety (168) and Wollman (155). Cerebral blood flow response to $PaCO_2$ values above 60 mm Hg diminished and at about a $PaCO_2$ value of 80 mm Hg, little or no increase occurred. Harper and Glass (42) obtained similar results in canine studies. Cerebral tissues showed a greater responsiveness to $PaCO_2$ change than did cerebellar or extracranial tissues and cerebral grey matter was more sensitive to $PaCO_2$ change than cerebral white matter.

Concurrent angiographical studies demonstrated that the more distal smaller vessels are more responsive to $PaCO_2$ change and correlated to a greater degree with CBF than the larger capacitance arteries. Furthermore, vessel diameter measurement did not provide and adequate index of the status of the cerebral circulation.

Subarachnoid hemorrhage induced during the normocapnic state produced a sustained global reduction (15 to 35 percent) in cerebral perfusion. Cerebral blood flow response to $PaCO_2$ change, although attenuated, was not abolished. Induced hypocapnia produced a further decrease in CBF in the post-SAH period while hypercapnia increased CBF (although not to the degree seen in the pre-SAH period). The CBF response curve was shifted to the right so that greater increases in $PaCO_2$ were necessary to achieve the level of response seen in the pre-SAH period. In general, CBF increased very gradually, or not at all, until $PaCO_2$ values of 60 mm Hg to 65 mm Hg were reached. Above $PaCO_2$ values of 60 mm Hg, a marked increase in cerebral perfusion occurred. This hypercapnia induced "CBF-breakthrough" phenomenon was demonstrated in all animals subjected to SAH. This phenomenon has not been previously described and its etiology can only be spectulated upon. Loss of autoregulation which is frequently present with hypercapnia (175) and also SAH, does not appear to be a factor responsible for the marked increase in CBF as blood pressure and heart rate remained essentially unchanged inthe post-SAH period. Perhaps extreme hypercapnia exerts its influence on the cerebral microcirculation. It is well recongnized that only a small portion of the cerebral microcirculation is patent during normal conditions (176). With extreme hypercapnia and loss of autoregulation, a greater portion of the microcirculation may be "opened" causing a marked increase in cerebral perfusion. Whether the increase in perfusion contributes to increased oxygen and glucose utilization and thus decreased morbidity remains to be investigated.

Angiographical studies demonstrated intradural vasospasm in all monkeys subjected to SAH. The degree of vasoconstriction varied among animals and to some extent, in different vessels in the same animal. Hypocapnia in the pos-SAH period increased the intensity of constriction, suggesting vessel reactivity was retained and SAH did not produce maximal constriction. Hypercapnia produced variable vessel caliber responses even though cerebral perfusion was always significantly increased. In one animal $PaCO_2$ increase produced a paradoxical response (intensified vasospasm) even though cerebral perfusion was significantly increased. DuBoulay (177) described a similar paradoxical response to hypercapnia in spastic arteries in patients suffering from SAH although CBF studies were not performed in his study. In a second animal $PaCO_2$ elevation caused mild relaxation of the spastic arteries. Even though intradural vasospasm was still evident during extreme hypercapnia, cerebral perfusion was increased above the pre-SAH normocapnia values and reflux of contrast medium into the opposite carotid and vertebral systems was abated. Similar findings (i.e. increased perfusion and decreased cerebrovascular resistance) were observed in the remaining hypercapnic animals displaying intradural vasospasm.

The effect of traumatic spasm on cerebral blood flow and intracranial vessel reactivity has been recently investigated in primates. Harper (178) studied CBF response to carbon dioxide increase prior to and following bilateral traumatic spasm of the internal carotid arteries. Cerebral blood flow response to $PaCO_2$ increase (from 40 mm Hg to 60 mm Hg) was markedly diminished in the post-spasm period.

Symon (179) demonstrated that hypocapnia (20 mm Hg) or hypercapnia (above 50 mm Hg) had no influence on the character or duration of spasm produced in the middle cerebral artery by traumatic stimulation. Spasm produced a decrease in perfusion pressure and cerebral perfusion in the peripheral distribution of the spastic artery and it was suggested that hypercapnia would, in fact, decrease perfusion in the ischemic area.

In the present study, traumatic spasm of one internal carotid artery produced a significant unilateral decrease in cerebral blood flow during normocapnia. Hypocapnia produced a further CBF decrease, suggesting preservation of the vasoconstrictor effect. Graded hypercapnia produced a corresponding increase in CBF which reached levels of significance at $PaCO_2$ values near 70 mm. Hg. Cerebral blood flow response to $PaCO_2$ increase was depressed as compared to the control studies and these results were in accord with Harper's work.

The present studies suggest that (i) SAH causes an increase in cerebrovascular resistance and a decrease in CBF by an ill-defined mechanism operating primarily at the microvascular level; (ii) hemodynamic responses to $PaCO_2$ change, although attenuated, are not abolished in the acute period after SAH. Hypocapnia decreased and hypercapnia increased CBF in the post-SAH period; (iii) hypercapnia ($PaCO_2 > 60$ mm Hg) significantly increased cerebral perfusion whether or not vasospasm was alleviated; and (iv) the small distal cerebral vessels (below radiological resolution) appear to be more reactive to $PaCO_2$ change and are more intimately associated with the regulation of cerebral perfusion.

Whether carbon dioxide inhalation therapy is of value in the treatment of cerebral ischemia caused by subarachnoid hemorrhage remains to be investigated. Chronic experimental studies would be essential for evaluating the effectiveness of intermittent or continuous carbon dioxide therapy on morbidity and mortality in animals subjected to SAH. The present results are encouraging and in highly selected, carefully managed patients, clinical trials may be warranted. Perhaps patients displaying evidence of cerebral ischemia after adequate clipping of a cerebral vessel aneurysm would be candidates for this form of therapy.

# CHAPTER SIX

## SUMMARY

Cerebral blood flow studies were performed in a total of 60 monkeys (3 related studies) utilizing the intra-arterial radio-isotope technique of Ingvar and Lassen. Control studies were performed in 19 animals, 31 animals were subjected to SAH, 4 animals received subarachnoid saline injection, 2 received subdural hemorrhage and 4 monkeys were subjected to traumatic spasm of one internal carotid artery. All animals were sedated with pentobartital sodium and anesthesia was maintained with a nitrous oxide-oxygen mixture. Other physiological parameters monitored included blood pressure, heart rate, arterial blood gases and pH, EKG and in several animals, EEG and intracranial pressure. Upon termination of the experiments, the animals were neurologically assessed and graded. Animals subjected to cerebral apoplexy were sacrificed for pathological verification of the lesions induced.

Although cerebral blood flow was calculated using the compartmental, stochastic and initial-slope-index methods, only the stochastic and initial-slope-index methods were utilized for statistical analysis.

In control animals cerebral blood flow and other physiological parameters remained constant over the 5 to 6 hour experimental period. Flow values recorded from four discrete supratentorial areas were not significantly different from each other and cerebellar flow was usually reduced by approximately 25 percent. Carbon dioxide inhalation caused a linear increase in CBF between $PaCO_2$ values of 30 mm Hg and 60 mm Hg and outside this range, CBF response was attenuated. Cerebral tissue showed a greater responsiveness to $PaCO_2$ change than cerebellar or extracranial tissues and grey matter was more sensitive to $PaCO_2$ change than cerebral white matter.

Subarachnoid hemorrhage caused a significant global reduction in cerebral perfusion in 75 to 80 percent of animals. The reduction was immediate and flow values remained significantly reduced for the duration of the experiments. Little variation in CBF occurred after reduced perfusion was established. Other physiological parameters were markedly altered during induction of SAH but these effects were transient (5-10 minutes). Animals with reduced cerebral blood flows after SAH displayed severe neurological deficits whereas those exhibiting no significant change in cerebral perfusion were healthy.

Angiographical vasospasm was present in all animals subjected to SAH. The spasm was generalized and persisted for the duration of the experiments. The degree of spasm present in animals exhibiting reduced cerebral perfusion was not significantly different from those with normal flows.

Cerebral insult (SAH and TSICA) caused a decreased cerebral blood flow response to carbon dioxide change. However, when $PaCO_2$ was raised to sufficiently high levels (60 mm Hg to 65 mm Hg), marked increases in cerebral perfusion occurred (Breakthough Phenomenon). Hypercapnia produced a significant increase in CBF even though cerebral vasospasm was not alleviated.

These studies also suggest that (i) cerebral vessels of a size below radiological resolution are important in the regulation of mainenance of perfusion; (ii) cerebral blood flow is more constantly related to clinical grade than is the degree of cerebral vasoconstriction after SAH; and (iii) measurement of CBF appears to be a more valuable tool as a prognostic indicator of cerebral function and survival than angiographical demonstration of arterial vasospasm.

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